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(54) Title: PEPTIDES AND RELATED MOLECULES THAT MODULATE NERVE GROWTH FACTOR ACTIVITY

(57) Abstract: The present invention relates to certain biologically active peptides and polypeptides which can be used as therapeutics or prophylactics against diseases or disorders linked to NGF as the causative agent. In one aspect of the present invention, pharmacologically active polypeptides comprising peptides linked to one or more Fc domains are provided.

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PEPTIDES AND RELATED MOLECULES THAT MODULATE
NERVE GROWTH FACTOR ACTIVITY

Background of the Invention

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More than two million people in the United States
alone are incapacitated by chronic pain on any given
day (T. M. Jessell & D. D. Kelly, Pain and Analgesia in
PRINCIPLES OF NEURAL SCIENCE, third edition (E. R.
10   Kandel, J. H. Schwartz, T. M. Jessell, ed., (1991)).
Unfortunately, current treatments for pain are only
partially effective, and many also cause debilitating
or dangerous side effects. For example, non-steroidal
anti-inflammatory drugs ("NSAIDs") such as aspirin,
15   ibuprofen, and indomethacin are moderately effective
against inflammatory pain but they are also renal
toxins, and high doses tend to cause gastrointestinal
irritation, ulceration, bleeding, and confusion.
Patients treated with opioids frequently experience
20   confusion, and long-term opioid use is associated with
tolerance and dependence. Local anesthetics such as
lidocaine and mixelitine simultaneously inhibit pain
and cause loss of normal sensation.

Pain is a perception based on signals received
25   from the environment and transmitted and interpreted by
the nervous system (for review, see Millan, M.J., The
induction of pain: an integrative review. Prog
Neurobiol 57:1-164 (1999)). Noxious stimuli such as
heat and touch cause specialized sensory receptors in
30   the skin to send signals to the central nervous system
("CNS"). This process is called nociception, and the
peripheral sensory neurons that mediate it are

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nociceptors. Depending on the strength of the signal
from the nociceptor(s) and the abstraction and
elaboration of that signal by the CNS, a person may or
may not experience a noxious stimulus as painful. When
5   one's perception of pain is properly calibrated to the
intensity of the stimulus, pain serves its intended
protective function. However, certain types of tissue
damage cause a phenomenon, known as hyperalgesia or
pronociception, in which relatively innocuous stimuli
10   are perceived as intensely painful because the person's
pain thresholds have been lowered. Both inflammation
and nerve damage can induce hyperalgesia. Thus,
persons afflicted with inflammatory conditions, such as
sunburn, osteoarthritis, colitis, carditis, dermatitis,
15   myositis, neuritis, collagen vascular diseases (which
include rheumatoid arthritis and lupus) and the like,
often experience enhanced sensations of pain.
Similarly, trauma, surgery, amputation, abscess,
causalgia, collagen vascular diseases, demyelinating
20   diseases, trigeminal neuralgia, cancer, chronic
alcoholism, stroke, thalamic pain syndrome, diabetes,
herpes infections, acquired immune deficiency syndrome
("AIDS"), toxins and chemotherapy cause nerve injuries
that result in excessive pain.
25   As the mechanisms by which nociceptors transduce
external signals under normal and hyperalgesic
conditions become better understood, processes
implicated in hyperalgesia can be targeted to inhibit
the lowering of the pain threshold and thereby lessen
30   the amount of pain experienced.

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Neurotrophic factors have been shown to play
significant roles in the transmission of physiologic
and pathologic pain. Nerve growth factor (NGF) appears
to be particularly important (for review, see McMahon,
5   S.B., NGF as a mediator of inflammatory pain, Phil
Trans R Soc Lond 351:431-40 (1996); and Apfel, S.C.,
Neurotrophic Factors and Pain, The Clinical Journal of
Pain 16:S7-S11 (2000)). Both local and systemic
administration of NGF have been shown to elicit
10   hyperalgesia and allodynia (Lewin, et al., Peripheral
and central mechanisms of NGF-induced hyperalgesia.
Eur. J. Neurosci. 6:1903-1912 (1994)). Intravenous
infusion of NGF in humans produces a whole body myalgia
while local administration evokes injection site
15   hyperalgesia and allodynia in addition to the systemic
effects (Apfel, et al., Recombinant human nerve growth
factor in the treatment of diabetic polyneuropathy.
Neurology 51: 695-702 (1998)). There is also a
considerable body of evidence implicating endogenous
20   NGF in conditions in which pain is a prominent feature.
For example, NGF is upregulated in DRG Schwann cells
for at least 2 months following peripheral nerve injury
and increased levels have been reported in the joints
of animals suffering from a variety of models of
25   arthritis (e.g., Aloe, et al., The synovium of
transgenic arthritic mice expressing human tumor
necrosis factor contains a high level of nerve growth
factor. Growth Factors 9:149-155 (1993)). In humans,
NGF levels are elevated in synovial fluid from patients
30   with rheumatoid or other types of arthritis (e.g., Aloe
et al., Nerve growth factor in the synovial fluid of

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patients with chronic arthritis. Arthritis and
Rheumatism 35:351-355 (1992)). Furthermore, it has been
demonstrated that antagonism of NGF function prevents
hyperalgesia and allodynia in models of neuropathic and
5   chronic inflammatory pain. For example, in models of
neuropathic pain (e.g. nerve trunk or spinal nerve
ligation) systemic injection of neutralizing antibodies
to NGF prevents both allodynia and hyperalgesia (Ramer,
M.S., et al., Adrenergic innervation of rat sensory
10   ganglia following proximal or distal painful sciatic
neuropathy: distinct mechanisms revealed by anti-NGF
treatment. Eur J Neurosci 11:837-846 (1999); and Ro,
L.S., et al., Effect of NGF and anti-NGF on neuropathic
pain in rats following chronic constriction injury of
15   the sciatic nerve. Pain 79: 265-274 (1999)).

Clearly, there is a need for new safe and
effective treatments for pain. It is an object of the
present invention to provide novel binding agents of
NGF that modulate NGF activity and that are useful for
20   managing pain. Such agents of the present invention
take the form of NGF binding peptides and NGF binding
modified peptides, i.e., peptides fused to other
molecules such as an Fc portion of an antibody, where
the peptide moiety specifically binds to NGF.

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Summary of the Invention

The present invention concerns novel agents which bind to and modulate the activity of nerve growth

5 factor (NGF).

In accordance with the present invention, modifiers of NGF activity comprise an amino acid sequence of the following formula:

$$a^1a^2a^3Ca^5a^6a^7a^8a^9a^{10}a^{11}LQSCa^{16}a^{17}a^{18}$$ (SEQ ID NO: 276)
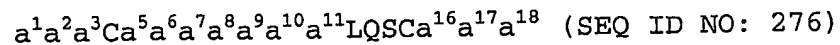
10 wherein:

a^1, a^2, a^3, a^5, a^6, a^8, and a^{18} are each independently absent or amino acid residues;

a^7 is a neutral hydrophobic or polar hydrophobic amino acid residue;

15 a^9 is a neutral hydrophobic or polar hydrophobic amino acid residue;

a^{10} is a neutral hydrophobic, neutral polar, or a basic amino acid residue;

a^{11} is a neutral hydrophobic, neutral polar, or a

20 basic amino acid residue;

a^{16} is a neutral hydrophobic amino acid residue;

a^{17} is a neutral hydrophobic or polar hydrophobic amino acid residue; or a physiologically acceptable salt thereof.

25 Also in accordance with the present invention are NGF activity modulating peptides comprising an amino acid sequence of the formula:

$$b^1b^2b^3CWb^6b^7b^8b^9GCb^{12}b^{13}b^{14}$$ (SEQ ID NO: 274)

wherein:

30 b^1, b^2, b^3, b^8, b^9, b^{13} and b^{14} are each independently absent or amino acid residues;

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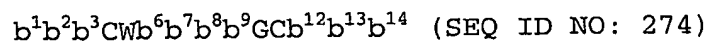
b^6 is a neutral hydrophobic amino acid residue;

b^7 is a polar hydrophobic amino acid residue;

b^{12} is a neutral hydrophobic or an acidic amino acid residue; or a physiologically acceptable salt thereof.

Further in accordance with the present invention are compounds of the formula:

$$c^1c^2QCc^5c^6Sc^8c^9GCc^{12}c^{13}c^{14}c^{15}c^{16}$$

wherein:

c^1, c^5, c^8, c^9, c^{13} and c^{14} are each independently absent or amino acid residues;

c^2 is a neutral hydrophobic amino acid residue;

c^6 is a neutral hydrophobic or polar hydrophopic amino acid residue;

c^{12} is a neutral hydrophobic or an acidic amino acid residue; or a physiologically acceptable salt thereof.

Further in accordance with the present invention are NGF activity modulating peptides comprising an amino acid sequence of the formula:

$$d^1d^2d^3d^4d^5d^6d^7PPd^{10}d^{11}d^{12}d^{13}d^{14}d^{15}Pd^{17}d^{18}d^{19}d^{20}d^{21}d^{22}d^{23}d^{24}$$

wherein:

d^1 is a W, Y, Q, or E;

d^2 is a V, L, F, S, or Q;

d^3 is a W, F, G, S, or Q;

d^4 is a A, Q, D, E, or K;

d^5 is a V, W, G, or R;

d^6 is a M, S, Y, Q, N, E, K, or R;

d^7 is a A, V, L, P, W, Q, or H;

d^{10} is a D or E;

d^{11} is a V or I;

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d^{12} is a V, L, F, or Y;

d^{13} is a V, L, G, Q, or E;

d^{14} is a Q, D, or E;

d^{15} is a W or C;

d^{17} is a W, Y, or Q;

d^{18} is a V, T, Q, N, or K;

d^{19} is a A, L, or P;

d^{20} is a P, Q, R, or H;

d^{21} is a V, I, W, D;

d^{22} is a A, I, S, Q, or D;

d^{23} is a L or absent;

d^{24} is a E or absent; or a physiologically acceptable salt thereof.

Other aspects of the invention are NGF activity modulating peptides comprising an amino acid sequence of the formula:

$$f^1f^2f^3f^4f^5f^6f^7f^8f^9f^{10}f^{11}Lf^{13}EQYFf^{18}Lf^{20}PPGf^{24}f^{25}f^{26}$$
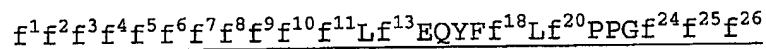
wherein:

f^1-f^6, f^8, f^9, f^{11}, f^{18}, f^{24}, f^{25} and f^{26} are each independently absent or amino acid residues;

f^7, f^{10}, and f^{13} are each independently neutral hydrophobic or polar hydrophopic amino acid residues;

f^{20} is a T, M, or I; or a physiologically acceptable salt thereof.

Further in accordance with the present invention are NGF activity modulating peptides comprising an amino acid sequence of the formula:

$$h^1h^2h^3h^4h^5h^6LGh^9h^{10}h^{11}Lh^{13}YFh^{16}Lh^{18}PPGh^{22}h^{23}h^{24}$$

wherein:

h^1-h^6, h^9, h^{11}, h^{23}, and h^{24} are each independently absent or amino acid residues;

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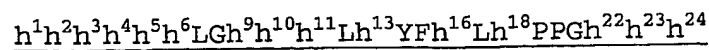
h^{10} and h^{13} are each independently neutral hydrophobic or polar hydrophopic amino acid residues;

h^{16} is a polar hydrophopic or basic amino acid residue;

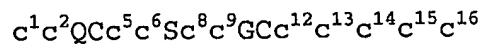h^{18} is a neutral hydrophopic amino acid residue;

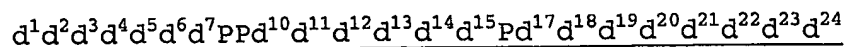h^{22} is a neutral polar amino acid residue; or a physiologically acceptable salt thereof.

Another aspect of the invention includes a pharmacologically active peptide (P) comprising an amino acid sequence selected from the group consisting of:

     i. SEQ ID NO: 1 to SEQ ID NO: 58, inclusive;

    ii. SEQ ID NO: 202 to SEQ ID NO: 280, inclusive;

    iii. an analog of (i) or (ii);

    iv. a derivative of (i), (ii) or (iii);

    v. a multimer of (i), (ii), (iii), or (iv); and

    vi. a physiologically acceptable salt of (i), (ii), (iii), (iv), or (v), wherein said peptide is capable of inhibiting NGF activity.

Another aspect of the invention comprises a modified peptide of the formula:

$$(X^1)_a - F^1 - (X^2)_b \qquad\qquad (I)$$

wherein:

F^1 is a vehicle (preferably an Fc domain);

X^1 and X^2 are each independently selected from $-(L^1)_c - P^1$, $-(L^1)_c - P^1 - (L^2)_d - P^2$, $-(L^1)_c - P^1 - (L^2)_d - P^2 - (L^3)_e - P^3$, and $-(L^1)_c - P^1 - (L^2)_d - P^2 - (L^3)_e - P^3 - (L^4)_f - P^4$;

L^1, L^2, L^3, and L^4 are each independently linkers;

a, b, c, d, e, and f are each independently 0 or 1, provided that at least one of a and b is 1; and

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P^1, P^2, P^3, and P^4 are each independently sequences of pharmacologically active peptides selected from the group consisting of:

 i. SEQ ID NO: 1 to SEQ ID NO: 58, inclusive;

5   ii. SEQ ID NO: 202 to SEQ ID NO: 280, inclusive;

  iii. an analog of (i) or (ii);

  iv. a derivative of (i), (ii) or (iii); and

  v. a physiologically acceptable salt of (i),

10 (ii), (iii), or (iv), wherein said peptide is capable of modulating NGF activity.

The peptides and modified peptides of the invention may be prepared by standard synthetic methods, recombinant DNA techniques, or any other

15 methods of preparing peptides and fusion proteins.

Modified peptides of the invention that encompass non-peptide portions may be synthesized by standard organic chemistry reactions, in addition to standard peptide chemistry reactions when applicable.

20 The peptides and modified peptides of the invention have therapeutic value for the treatment of chronic pain states of neuropathic or inflammatory origin, and can also be used to treat other diseases linked to NGF as a causative agent, including, but not

25 limited to, migraine, asthma, urge incontinence (i.e., hyperactive bladder), psoriasis, and cancer (especially, pancreatic cancer and melanoma).

The peptides and modified peptides of the invention may be used for therapeutic or prophylactic

30 purposes by formulating them with appropriate pharmaceutical carrier materials and administering an

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effective amount to a patient, such as a human (or
other mammal) in need thereof.

Additional useful peptides and modified peptides
may result from conservative modifications of the amino
5   acid of the peptides and modified peptides disclosed
herein. Conservative modifications will produce
peptides and modified peptides having functional,
physical, and chemical characteristics similar to those
of the peptide or modified peptide from which such
10   modifications are made.

Additional aspects and advantages of the present
invention will become apparent upon consideration of
the detailed description of the invention which
follows.

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Brief Description of the Figures

Figure 1 shows the structure of a typical or
preferred modified peptide of the invention. "Fc" in
5   this figure represents any of the Fc variants within
the meaning of "Fc domain" herein. The modified
peptide is comprised of a homodimer comprised of two Fc
monomers, each with one attached peptide. The purified
"dimer" possesses twelve cysteine residues which form
10   two intermolecular and four intramolecular disulfide
bonds as depicted. Figure 1A shows a molecule in which
the linker-peptide portion is present as single chains
extending from the N-terminus of the Fc domain. Figure
1B shows a molecule in which the linker-peptide portion
15   is present as single chains extending from the C-
terminus of the Fc domain.

Figure 2 shows exemplary nucleic acid and amino
acid sequences (SEQ ID NOS: 59 and 60, respectively) of
human IgG1 Fc that may be used in the invention.

20   Figure 3A-B shows the double stranded DNA sequence
(SEQ ID NOs: 61 and 62, top/sense and bottom/anti-sense
strands, respectively) of an Fc N-terminal vector
inserted into expression plasmid pAMG21 between the
NdeI restriction site (position #5675 in pAMG21) and
25   BamHI restriction site (position #5745 in pAMG21),
resulting in an expression plasmid capable of
expressing peptide-Fc fusion proteins in accordance
with the invention.

Figure 4A-B shows the double stranded DNA sequence
30   (SEQ ID NOS: 121 and 122, top/sense and bottom/anti-
sense strands, respectively) of an Fc C-terminal vector
inserted into expression plasmid pAMG21 between the

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NdeI restriction site (position #5675 in pAMG21) and
BamHI restriction site (position #5745 in pAMG21),
resulting in an expression plasmid capable of
expressing peptide-Fc fusion proteins in accordance
5   with the invention.

Figure 5 depicts graphs of the antiallodynic
effects of anti-NGF modified peptides (A-H as described
in Table 6) in Chung neuropathic pain model in rats (60
mg/kg, s.c., at day 3 or 4 after administration).

10   Figure 6 depicts graphs of the antiallodynic
effects of anti-NGF modified peptides (I-J as described
in Table 6) in CFA inflammatory pain model in rats (60
mg/kg, s.c., at day 3 or 4 after administration).

15   Detailed Description of the Invention

The terms used throughout this specification are
defined as follows, unless otherwise limited in
specific instances.

20   Amino acid residues are discussed in three ways:
full name of the amino acid, standard three-letter
code, or standard single-letter code in accordance with
the chart shown below.

A = Ala	G = Gly	M = Met	S = Ser
C = Cys	H = His	N = Asn	T = Thr
D = Asp	I = Ile	P = Pro	V = Val
E = Glu	K = Lys	Q = Gln	W = Trp
F = Phe	L = Leu	R = Arg	Y = Tyr

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The term "comprising" means that a peptide or
modified peptide may include additional amino acids on
either or both of the N- or C- termini of the given
sequence. Of course, these additional amino acids

5   should not significantly interfere with the activity of
the peptide or modified peptide.

Modifications can protect therapeutic peptides and
proteins, primarily by blocking their exposure to
proteolytic enzymes, leading to increased stability,

10   circulation time and biological activity of the
therapeutic molecule. A review article describing
protein modification and fusion proteins is Francis,
Focus on Growth Factors Volume 3, pages 4-10, published
by Mediscript, London (1992), which is hereby

15   incorporated by reference.

One useful protein modification is a combination
with the "Fc" domain of an antibody. Antibodies
comprise two functionally independent parts, a variable
domain known as "Fab", which binds antigen, and a

20   constant domain known as "Fc", which links to such
effector functions as complement activation and attack
by phagocytic cells. An Fc domain has a long serum
half-life, whereas a Fab is short-lived. Capon et al.,
Nature, Volume 337, pages 525-31 (1989). When

25   constructed together with a therapeutic protein, an Fc
domain can provide longer half-life or incorporate such
functions as Fc receptor binding, protein A binding,
complement fixation, and perhaps even placental
transfer.

30   As used herein, the term "native Fc" refers to a
molecule or sequence comprising the sequence of a non-

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antigen-binding fragment resulting from chemical or
enzymatic digestion of whole antibody. The original
immunoglobulin source of the native Fc is preferably of
human origin and may be any of the immunoglobulins,
5   although IgG1 and IgG2 are preferred. Native Fc
domains are made up of monomeric polypeptides that may
be linked into dimeric or multimeric forms by covalent
(i.e., disulfide bonds) and non-covalent association.
The number of intermolecular disulfide bonds between
10   monomeric subunits of native Fc molecules ranges from 1'
to 4 depending on class (e.g., IgG, IgA, IgE) or
subclass (e.g., IgG1, IgG2, IgG3, IgA1, IgGA2). One
example of a native Fc is a disulfide-bonded dimer
resulting from papain digestion of an IgG; see Ellison
15   et al., Nucleic Acids Res., Volume 10, pages 4071-4079
(1982).

        The term "Fc variant" refers to a molecule or
sequence that is modified from a native Fc but still
comprises a binding site for the salvage receptor,
20   FcRn. International applications WO 97/34631 and
WO 96/32478 describe exemplary Fc variants, as well as
interaction with the salvage receptor, and are hereby
incorporated by reference. Thus, the term "Fc variant"
comprises a molecule or sequence that is humanized from
25   a non-human native Fc. Furthermore, a native Fc
comprises sites that may be removed because they
provide structural features or biological activity that
are not required for the fusion molecules of the
present invention. Thus, the term "Fc variant"
30   comprises a molecule or sequence that lacks one or more
native Fc sites or residues that affect or are involved

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in (1) disulfide bond formation, (2) incompatibility
with a selected host cell (3) N-terminal heterogeneity
upon expression in a selected host cell, (4)
glycosylation, (5) interaction with complement, (6)

5   binding to an Fc receptor other than a salvage
receptor, or (7) antibody-dependent cellular
cytotoxicity (ADCC). Fc variants are described in
further detail hereinafter.

The term "Fc domain" and the term "Fc" are

10   intended to encompass native Fc and Fc variant
molecules and sequences as defined above.

Published International Patent Application
WO 00/24782 describes fusion proteins comprising Fc
antibody domains linked to biologically active peptides

15   and their use as pharmaceutical agents. Linkage of the
peptides to the Fc domains is disclosed as increasing
the half-life of the peptide, which would otherwise be
quickly degraded in vivo. The peptides can be selected
by phage display, E. coli display, ribosome display,

20   RNA-peptide screening or chemical-peptide screening.
Specifically exemplified are Fc fusion products made
from peptide mimetics of TPO (megakaryocyte growth and
differentiation factor) and peptide inhibitors of
TNF-α, IL-1 and VEGF, among others.

25   The terms "derivatizing" and "derivative" or
"derivatized" comprise processes and resulting peptides
or modified peptides, respectively, in which (1) the
peptide or modified peptide has a cyclic portion; for
example, cross-linking between cysteinyl residues

30   within the modified peptide; (2) the peptide or
modified peptide is cross-linked or has a cross-linking

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site; for example, the peptide or modified peptide has
a cysteinyl residue and thus forms cross-linked dimers
in culture or *in vivo*; (3) one or more peptidyl linkage
is replaced by a non-peptidyl linkage; (4) the N-
5   terminus is replaced by $-NRR^1$, $NRC(O)R^1$, $-NRC(O)OR^1$, $-NRS(O)_2R^1$, $-NHC(O)NHR$, a succinimide group, or
substituted or unsubstituted benzyloxycarbonyl-NH-,
wherein R and R^1 and the ring substituents are as
defined hereinafter; (5) the C-terminus is replaced by
10   $-C(O)R^2$ or $-NR^3R^4$ wherein R^2, R^3 and R^4 are as defined
hereinafter; and (6) peptides or modified peptides in
which individual amino acid moieties are modified
through treatment with agents capable of reacting with
selected side chains or terminal residues. Derivatives
15   are further described hereinafter.

        The term "NGF" means nerve growth factor.

        The interaction of a protein ligand with its
receptor often takes place at a relatively large
interface. However, as demonstrated for human growth
20   hormone and its receptor, only a few key residues at
the interface contribute to most of the binding energy.
Clackson et al., Science, Volume 267, pages 383-386
(1995). The bulk of the protein ligand merely displays
the binding epitopes in the right topology or serves
25   functions unrelated to binding. Thus, molecules of
only "peptide" length can bind to the receptor protein
of a given large protein ligand. Such peptides may
mimic the bioactivity of the large protein ligand
("peptide agonists") or, through competitive binding,
30   inhibit the bioactivity of the large protein ligand
("peptide antagonists").

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The term "peptide" as used generally herein refers
to molecules of 5 to 50 amino acids, with molecules of
5 to 20 amino acids being preferred and those of 6 to
15 amino acids being most preferred.

5   Phage display peptide libraries have emerged as a
powerful method in identifying peptide agonists and
peptide antagonists. See, for example, Scott et al.,
Science, Volume 249, page 386 (1990); Devlin et al.
Science, Volume 249, page 404 (1990); U.S. Patent No.
10 5,223,409, issued June 29, 1993; U.S. Patent No.
5,733,731, issued March 31, 1998; U.S. Patent No.
5,498,530, issued March 12, 1996; U.S. Patent No.
5,432,018, issued July 11, 1995; U.S. Patent No.
5,338,665, issued August 16, 1994; U.S. Patent No.
15 5,922,545, issued July 13, 1999; WO 96/40987, published
December 19, 1996; and WO 98/15833, published April 16,
1998 (each of which is incorporated herein by
reference). In such libraries, random peptide
sequences are displayed by fusion with coat proteins of
20 filamentous phage. Typically, the displayed peptides
are affinity-eluted against an antibody-immobilized
extracellular domain of a receptor. The retained
phages may be enriched by successive rounds of affinity
purification and repropagation. The best binding
25 peptides may be sequenced to identify key residues
within one or more structurally related families of
peptides. See, for example, Cwirla et al., Science
Volume 276, pages 1696-1699 (1997), in which two
distinct families were identified. The peptide
30 sequences may also suggest which residues may be safely
replaced by alanine scanning or by mutagenesis at the

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DNA level. Mutagenesis libraries may be created and
screened to further optimize the sequence of the best
binders. Lowman, Ann. Rev. Biophys. Biomol. Struct.,
Volume 26, pages 401-424 (1997).

5   Structural analysis of protein-protein interaction
may also be used to suggest peptides that mimic the
binding activity of large protein ligands. In such an
analysis, the crystal structure may suggest the
identity and relative orientation of critical residues

10 of the large protein ligand from which a peptide may be
designed. See, for example, Takasaki et al., Nature
Biotech., Volume 15, pages 1266-1270 (1997). These
analytical methods may also be used to investigate the
interaction between a receptor protein and peptides

15 selected by phage display, which may suggest further
modification of the peptides to increase binding
affinity.

        Other methods compete with phage display in
peptide research. A peptide library can be fused to

20 the carboxyl terminus of the *lac* repressor and
expressed in *E. coli*. Another *E. coli*-based method
allows display on the outer membrane of the cell by
fusion with a peptidoglycan-associated lipoprotein
(PAL). Hereinafter, these and related methods are

25 collectively referred to as "*E. coli* display." In
another method, translation of random RNA is halted
prior to ribosome release, resulting in a library of
polypeptides with their associated RNA still attached.
Hereinafter, this and related methods are collectively

30 referred to as "ribosome display." Still other methods
employ chemical linkage of peptides to RNA; see, for

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example, Roberts and Szostak, Proc. Natl. Acad. Sci.
USA, Volume 94, pages 12297-12303 (1997). Hereinafter,
this and related methods are collectively referred to
as "RNA-peptide screening." Chemically derived peptide
5   libraries have also been developed in which peptides
are immobilized on stable, non-biological materials,
such as polyethylene rods or solvent-permeable resins.
Another chemically derived peptide library uses
photolithography to scan peptides immobilized on glass
10   slides. Hereinafter, these and related methods are
collectively referred to as "chemical-peptide
screening." Chemical-peptide screening may be
advantageous in that it allows use of D-amino acids and
other unnatural analogs, as well as non-peptide
15   elements. Both biological and chemical methods are
reviewed in Wells and Lowman, Curr. Opin. Biotechnol.,
Volume 3, pages 355-362 (1992).

Conceptually, one may discover peptide mimetics of
any protein using phage display, and the other methods
20   mentioned above. These methods have been used for
epitope mapping, for identification of critical amino
acids in protein-protein interactions, and also as
leads for the discovery of new therapeutic agents. For
example, see Cortese et al., Curr. Opin. Biotech.
25   Volume 7, pages 616-621 (1996). Peptide libraries are
now being used most often in immunological studies,
such as epitope mapping. Kreeger, The Scientist,
Volume 10, Number 13, pages 19-20 (1996). Peptides are
oftentimes regarded as "leads" in development of
30   therapeutic agents rather than as therapeutic agents
themselves. Like many other proteins, they would be

20

rapidly removed *in vivo* by renal filtration, cellular

clearance mechanisms in the reticulo-endothelial

system, or proteolytic degradation. See Francis, Focus

on Growth Factors, Volume 3, pages 4-11 (1992). As a

5   result, the identified peptides are often used to

validate drug targets or as scaffolds for design of

organic modified peptides that might not have been as

easily or as quickly identified through chemical

library screening. Lowman, Ann. Rev. Biophys. Biomol.

10   Struct., Volume 26, pages 401-424 (1997); Kay et al.,

Drug Disc. Today, Volume 3, pages 370-378(1998).

The term "pharmacologically active" means that a

substance so described is determined to have activity

that affects a medical parameter or disease state (for

15   example, pain). In the context of the invention, this

term typically refers to an NGF-induced or NGF-mediated

disease or abnormal medical condition or disorder, and

more specifically, to antagonism of pain.

The terms "antagonist" and "inhibitor" refer to a

20   molecule that blocks or in some way interferes with the

biological activity of the associated protein of

interest. A preferred "antagonist" or "inhibitor" of

the present invention is a molecule that binds to and

inhibits NGF with an IC_{50} of 20 nM or less in *in vitro*

25   assays of NGF activity. A more preferred "antagonist"

or "inhibitor" of the present invention is a molecule

that binds to and inhibits NGF with an IC_{50} of 1 nM or

less in *in vitro* assays of NGF activity. A most

preferred "antagonist" or "inhibitor" of the present

30   invention is a molecule that binds to and inhibits NGF

with an IC_{50} of 20 nM or less in *in vitro* assays of NGF

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activity and prevents, ameliorates or abolishes pain as measured in at least one generally accepted *in vivo* animal model of neurological pain.

Additionally, physiologically acceptable salts of

5   the modified peptides of the invention are also encompassed herein. By "physiologically acceptable salts" is meant any salts that are known or later discovered to be pharmaceutically acceptable (i.e., useful in the treatment of a warm-blooded animal).

10   Some specific examples are: acetate; trifluoroacetate; hydrohalides, such as hydrochloride and hydrobromide; sulfate; citrate; tartrate; glycolate; and oxalate.

Structure of modified peptides

15

In General. With respect to the modified peptides of the present invention, the peptide portion may be attached to the vehicle (i.e., Fc domain) through the N-terminus and/or C-terminus of the peptide. Thus, the

20   resulting vehicle-peptide composite may be described by the following formula:

$$(X^1)_a-F^1-(X^2)_b \qquad\qquad (I)$$

wherein:

F^1 is a vehicle (preferably an Fc domain);

25   X^1 and X^2 are each independently selected from $-(L^1)_c-P^1$, $-(L^1)_c-P^1-(L^2)_d-P^2$, $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$, and $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$;

L^1, L^2, L^3, and L^4 are each independently linkers;

a, b, c, d, e, and f are each independently 0 or

30   1, provided that at least one of a and b is 1; and

22

P^1, P^2, P^3, and P^4 are each independently a
pharmacologically active peptide selected from the
group consisting of:

      i. SEQ ID NO: 1 to SEQ ID NO: 58, inclusive;

5   ii. SEQ ID NO: 202 to SEQ ID NO: 280,
inclusive;

      iii. an analog of (i) or (ii); and

      iv. a derivative of (i), (ii) or (iii).

10   The modified peptides of formula I will comprise
preferred embodiments of the formulas:

$$X^1-F^1 \qquad\qquad (II)$$

wherein F^1 is an Fc domain and is attached at the
C-terminus of X^1;

15 $$F^1-X^2 \qquad\qquad (III)$$
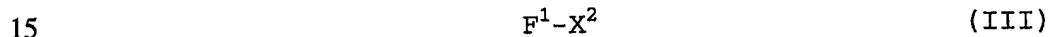
wherein F^1 is an Fc domain and is attached at the
N-terminus of X^2; and

$$F^1-(L)_c-P \qquad\qquad (IV)$$
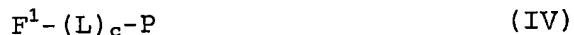
wherein F^1 is an Fc domain and is attached at the
20 N-terminus of -(L)$_c$-P.

In addition to the peptides (P) and the modified
peptides provided by formulas (I)-(IV), also intended
as part of the invention are fragments (i.e.,
"subsequences"), analogs, and derivatives of such
25 peptides and modified peptides which are substantially
equivalent with respect to *in vitro and/or in vivo*
anti-NGF activity, including but not limited to,
monomers or multimers of any of the peptides (P)
disclosed herein.

30   The term "analog" is intended to mean molecules
representing one or more amino acid substitutions,

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deletions and/or additions derived from the linear
array of amino acids of the peptides (P) or the
modified peptides provided for by (I)-(IV), and which
are substantially equivalent with respect to *in vitro*
5   and/or *in vivo* anti-NGF activity as compared to at
least one analogous peptide or modified peptide
specifically disclosed herein.

For the purposes of the invention, "substantially
homologous" sequences are at least 81%, preferably at
10   least 85%, more preferably at least 90%, and most
preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%,
98%, 99% identical, over any region of P of at least 10
amino acids, as determined by any of the alignment
methods generally applied in the art (for example, the
15   GAP program) and/or as discussed herein, even if the
sequences differ more substantially outside of the P
region.

Percent sequence identity can be determined by
standard methods that are commonly used to compare the
20   similarity in position of the amino acids of two
polypeptides in order to generate an optimal alignment
of two respective sequences. Such methods include, but
are not limited to, those described in Computational
Molecular Biology, Lesk, A.M., ed., Oxford University
25   Press, New York, 1988; Biocomputing: Informatics and
Genome Projects, Smith, D.W., ed., Academic Press, New
York, 1993; Computer Analysis of Sequence Data, Part 1,
Griffin, A.M., and Griffin, H.G., eds., Humana Press,
New Jersey, 1994; Sequence Analysis in Molecular
30   Biology, von Heinje, G., Academic Press, 1987; Sequence
Analysis Primer, Gribskov, M. and Devereux, J., eds.,

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M. Stockton Press, New York, 1991; and Carillo *et al.*,
SIAM J. Applied Math., 48:1073 (1988). Methods to
determine identity and similarity are also described in
publicly available computer programs. Preferred
5   computer program methods to determine identity and
similarity between two sequences include, but are not
limited to, the GCG program package, including GAP
(Devereux *et al.*, *Nucl. Acid. Res.*, 12:387 (1984);
Genetics Computer Group, University of Wisconsin,
10   Madison, WI), BLASTP, BLASTN, and FASTA (Altschul *et
al.*, *J. Mol. Biol.*, 215:403-410 (1990)). The BLASTX
program is publicly available from the National Center
for Biotechnology Information (NCBI) and other sources
(*BLAST Manual,* Altschul *et al.* NCB/NLM/NIH Bethesda, MD
15   20894; Altschul *et al., supra*). The well known Smith
Waterman algorithm may also be used to determine
identity.

Certain alignment schemes for aligning two amino
acid sequences may result in the matching of only a
20   short region of the two sequences, and this small
aligned region may have very high sequence identity
even though there is no significant relationship
between the two full length sequences. Preferred
methods to determine identity and/or similarity are
25   designed to give the largest match between the
sequences tested. For example, using the computer
algorithm GAP (Genetics Computer Group, University of
Wisconsin, Madison, WI), two polypeptides for which the
percent sequence identity is to be determined are
30   aligned for optimal matching of their respective amino
acids (the "matched span", as determined by the

25

algorithm). A gap opening penalty (which is calculated
as 3X the average diagonal; the "average diagonal" is
the average of the diagonal of the comparison matrix
being used; the "diagonal" is the score or number
5   assigned to each perfect amino acid match by the
particular comparison matrix) and a gap extension
penalty (which is usually 1/10 times the gap opening
penalty), as well as a comparison matrix such as
PAM 250 or BLOSUM 62 are used in conjunction with the
10   algorithm. A standard comparison matrix (see Dayhoff
et al., Atlas of Protein Sequence and Structure, vol.
5, supp.3 (1978) for the PAM 250 comparison matrix;
Henikoff et al., Proc. Natl. Acad. Sci USA, 89:10915-
10919 (1992) for the BLOSUM 62 comparison matrix) is
15   also used by the algorithm. Preferred parameters for a
polypeptide sequence comparison include the following:

Algorithm: Needleman et al., J. Mol. Biol.,
48:443-453 (1970);
Comparison matrix: BLOSUM 62 from Henikoff et
20   al., Proc. Natl. Acad. Sci. USA, 89:10915-10919
(1992);
Gap Penalty: 12
Gap Length Penalty: 4
Threshold of Similarity: 0

25

The GAP program is useful with the above
parameters. The aforementioned parameters are the
default parameters for polypeptide comparisons (along
with no penalty for end gaps) using the GAP algorithm.
30   Preferred parameters for nucleic acid molecule
sequence comparisons include the following:

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Algorithm: Needleman *et al.*, *J. Mol Biol.*, 48:443-
453 (1970);
Comparison matrix: matches = +10, mismatch = 0
Gap Penalty: 50
5   Gap Length Penalty: 3

The GAP program is also useful with the above
parameters. The aforementioned parameters are the
default parameters for nucleic acid molecule
10   comparisons.
Other exemplary algorithms, gap opening penalties,
gap extension penalties, comparison matrices,
thresholds of similarity, etc. may be used, including
those set forth in the Program Manual, Wisconsin
15   Package, Version 9, September, 1997. The particular
choices to be made will be apparent to those of skill
in the art and will depend on the specific comparison
to be made, such as DNA to DNA, protein to protein,
protein to DNA; and additionally, whether the
20   comparison is between given pairs of sequences (in
which case GAP or BestFit are generally preferred) or
between one sequence and a large database of sequences
(in which case FASTA or BLASTA are preferred).
Using a known computer program such as BLAST or
25   FASTA, two polypeptides are aligned for optimal
matching of their respective amino acids (either along
the full length of one or both sequences, or along a
pre-determined portion of one or both sequences). The
programs provide a "default" opening penalty and a
30   "default" gap penalty, and a scoring matrix such as PAM
250. A standard scoring matrix can be used in

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conjunction with the computer program; see Dayhoff et

al., in Atlas of Protein Sequence and Structure, volume

5, supplement 3 (1978). The percent identity can then

be calculated as follows:

<u>Total number of identical matches   X 100</u>
[No. of residues in region of alignment, not
including non-identical residues at either or
both ends and residues opposite a gap]

5   Analog polypeptides in accordance with the

invention will typically have one or more amino acid

substitutions, deletions and/or insertions. It is

generally recognized that conservative amino acid

changes are least likely to perturb the structure

10   and/or function of a polypeptide and generally involve

substitution of one amino acid with another that is

similar in structure and/or function (e.g., amino acids

with side chains similar in size, charge and/or shape).

The nature of these substitutions are well known to one

15   skilled in the art and exemplary amino acid

substitutions are summarized in Tables 1 and 2.

Table 1: Amino Acid Substitutions

Basic:
Arg; Lys; His; Asn; Gln

Acidic:
Glu; Asp

Polar:
Glu; Asn

Hydrophilic:
Asp; Glu; Asn; Ser; Tyr

Hydrophobic:
Ala; Met; Ile; Leu; nor-Leu; Val

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Aromatic:
Phe; Trp; Tyr

Small:
Gly; Ala; Ser; Thr; Met

29

Table 2: Amino Acid Substitutions

Amino Acid	Preferred Substitutions	Most Preferred Substitution
Ala	Gly; Leu; Ile; Asn; Pro	Val
Arg	Ala; Asn; Gln; Ser	Lys
Asn	Arg; Gln; His; Lys; Ser; Tyr	Gln
Asp	Asn; Ser; Thr; Gln	Glu
Cys	Ala	Ser
Gln	Ala; Arg; Glu; Leu; Lys; Met; Ser; Tyr	Asn
Glu	Gln; Ser; Thr; Asn	Asp
Gly		Pro
His	Asn; Gln; Lys; Tyr; Phe	Arg
Ile	Tyr; Val; Met; Ala; Phe; nor-Leu	Leu
Leu	nor-Leu; Ile; Val; Met; Ala; Phe	Ile
Lys	Asn; Asp; Ala; Glu; Gln; Ser; Tyr	Arg
Met	Ala; Gln; Tyr; Trp; Phe	Leu
Phe	Leu; Val; Ile; Ala; Met	Leu
Pro	Ile; Val	Gly
Ser	Ala; Asn; Asp; Gly; Lys	Thr
Thr	Ala; Gly; Ile; Val; Lys	Ser
Trp	Phe; Tyr; His	Tyr
Tyr	Trp; Thr; Ser	Phe
Val	Ala; Ile; Met; Phe; Tyr; nor-Leu	Leu

5   Changing from A, F, H, I, L, M, P, V, W, or Y to C is
more preferred if the new cysteine remains as a free
thiol.

     Desired amino acid substitutions (whether

10 conservative or non-conservative) can be determined by
those skilled in the art at the time such substitutions
are desired. For example, amino acid substitutions can
be used to identify important residues of the peptide
sequence, or to increase or decrease the affinity of

30

the peptide or vehicle-peptide molecules (see preceding formula) described herein.

In certain embodiments, conservative amino acid substitutions also encompass non-naturally occurring
5 amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems.

As noted in the foregoing section, naturally occurring residues may be divided into classes based on
10 common side chain properties that may be useful for modifications of sequence. For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be
15 introduced into regions of the peptide that are homologous with non-human orthologs, or into the non-homologous regions of the molecule. In addition, one may also make modifications using P or G for the purpose of influencing chain orientation.
20   In making such modifications, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine
25 (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline   (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5);
30 asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

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The importance of the hydropathic amino acid index
in conferring interactive biological function on a
protein is understood in the art. Kyte et al., J. Mol.
Biol., 157: 105-131 (1982). It is known that certain
5   amino acids may be substituted for other amino acids
having a similar hydropathic index or score and still
retain a similar biological activity. In making
changes based upon the hydropathic index, the
substitution of amino acids whose hydropathic indices
10 are within ±2 is preferred, those which are within ±1
are particularly preferred, and those within ±0.5 are
even more particularly preferred.

It is also understood in the art that the
substitution of like amino acids can be made
15 effectively on the basis of hydrophilicity. The
greatest local average hydrophilicity of a protein, as
governed by the hydrophilicity of its adjacent amino
acids, correlates with its immunogenicity and
antigenicity, i.e., with a biological property of the
20 protein.

The following hydrophilicity values have been
assigned to amino acid residues: arginine (+3.0);
lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ±
1); serine (+0.3); asparagine (+0.2); glutamine (+0.2);
25 glycine (0); threonine (-0.4); proline (-0.5 ± 1);
alanine (-0.5); histidine (-0.5); cysteine (-1.0);
methionine (-1.3); valine (-1.5); leucine (-1.8);
isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-
2.5); tryptophan (-3.4). In making changes based upon
30 similar hydrophilicity values, the substitution of
amino acids whose hydrophilicity values are within ±2

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is preferred, those which are within ±1 are
particularly preferred, and those within ±0.5 are even
more particularly preferred. One may also identify
epitopes from primary amino acid sequences on the basis
5   of hydrophilicity. These regions are also referred to
as "epitopic core regions."

   A skilled artisan will be able to determine
suitable analogs of the peptides and modified peptides
set forth herein using well known techniques. For
10 identifying suitable areas of the molecule that may be
changed without destroying activity, one skilled in the
art may target areas not believed to be important for
activity. It will be appreciated that changes in areas
of a peptide that are not conserved relative to other
15 such similar peptides would be less likely to adversely
affect the biological activity and/or structure of the
peptide. One skilled in the art would also know that,
even in relatively conserved regions, one may
substitute chemically similar amino acids for the
20 naturally occurring residues while retaining activity
(conservative amino acid residue substitutions).
Therefore, even areas that may be important for
biological activity or for structure may be subject to
conservative amino acid substitutions without
25 destroying the biological activity or without adversely
affecting the peptide structure.

   Additionally, one skilled in the art can review
structure-function studies identifying residues in
similar peptides that are important for activity or
30 structure. In view of such a comparison, one can
predict the importance of amino acid residues in a

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peptide that correspond to amino acid residues that are
important for activity or structure in similar
peptides. One skilled in the art may opt for
chemically similar amino acid substitutions for such
5   predicted important amino acid residues of the peptides
or modified peptides of the present invention.

One skilled in the art can also analyze the three-
dimensional structure and amino acid sequence in
relation to that structure in similar peptides or
10   polypeptides. In view of that information, one skilled
in the art may predict the alignment of amino acid
residues of a peptide or a polypeptide with respect to
its three dimensional structure. One skilled in the
art may choose not to make radical changes to amino
15   acid residues predicted to be on the surface of the
protein, since such residues may be involved in
important interactions with other molecules. Moreover,
one skilled in the art may generate test analogs
containing a single amino acid substitution at each
20   desired amino acid residue. The analogs can then be
screened using activity assays know to those skilled in
the art. Such data could be used to gather information
about suitable analogs. For example, if one discovered
that a change to a particular amino acid residue
25   resulted in destroyed, undesirably reduced, or
unsuitable activity, analogs with such a change would
be avoided. In other words, based on information
gathered from such routine experiments, one skilled in
the art can readily determine the amino acids where
30   further substitutions should be avoided either alone or
in combination with other mutations.

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A number of scientific publications have been
devoted to the prediction of secondary structure. See
Moult J., Curr. Op. in Biotech., 7(4):422-427 (1996),
Chou et al., Biochemistry, 13(2):222-245 (1974); Chou
5   et al., Biochemistry, 113(2):211-222 (1974); Chou et
al., Adv. Enzymol. Relat. Areas Mol. Biol., 47:45-148
(1978); Chou et al., Ann. Rev. Biochem., 47:251-276 and
Chou et al., Biophys. J., 26:367-384 (1979). Moreover,
computer programs are currently available to assist
10   with predicting secondary structure. One method of
predicting secondary structure is based upon homology
modeling. For example, two polypeptides or proteins
which have a sequence identity of greater than 30%, or
similarity greater than 40% often have similar
15   structural topologies. The recent growth of the
protein structural data base (PDB) has provided
enhanced predictability of secondary structure,
including the potential number of folds within a
polypeptide's or protein's structure. See Holm et al.,
20   Nucl. Acid. Res., 27(1):244-247 (1999). It has been
suggested (Brenner et al., Curr. Op. Struct. Biol.,
7(3):369-376 (1997)) that there are a limited number of
folds in a given polypeptide or protein and that once a
critical number of structures have been resolved,
25   structural prediction will gain dramatically in
accuracy.
     Additional methods of predicting secondary
structure include "threading" (Jones, D., Curr. Opin.
Struct. Biol., 7(3):377-87 (1997); Sippl et al.,
30   Structure, 4(1): 15-9 (1996)), "profile analysis"
     (Bowie et al., Science, 253:164-170 (1991); Gribskov et

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al., Meth. Enzym., 183:146-159 (1990); Gribskov et al.,
Proc. Nat. Acad. Sci., 84(13):4355-8 (1987)), and
"evolutionary linkage" (See Home, supra, and Brenner,
supra).

5   Peptide and modified peptide fragments (i.e.,
subsequences) included within the invention will be
those that have less than the full length sequence, but
which possess substantially the same biological
activity *in vitro and/or in vivo* with respect to anti-
10   NGF activity and are truncated at the amino terminus,
the carboxy terminus, and/or internally.

        Peptide and modified peptide analogs, fragments,
and derivatives in accordance with the invention will
be useful for the same purposes for which the peptides
15   and modified peptides specifically disclosed herein are
useful (i.e., antagonists of NGF activity *in vitro*
and/or *in vivo*).

Peptides. The peptides used in conjunction with the
20   present invention are, as mentioned, peptides that
modulate (e.g., increase or decrease) the activity of
NGF. Phage display, in particular, has been useful in
generating the peptides which are listed below in Table
3 (SEQ ID NOS: 1-29). Also useful are the methionyl-
25   mature (Met^{-1}) versions of each of these peptides, in
which a methionine residue may be expressed at the N-
terminus (SEQ ID NOS: 30-58). Especially preferred
peptides of the present invention are the affinity
matured peptides listed below in Table 5 (SEQ ID NOS:
30   202-280).

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Vehicles. The term "vehicle" as used herein refers to
a molecule that prevents degradation and/or increases
half-life, reduces toxicity, reduces immunogenicity, or
increases biological activity of a therapeutic protein.

5   In the context of the invention, the preferred vehicle
constitutes an Fc domain. One aspect of the invention
requires the presence of at least one vehicle (F^1)
attached to a peptide through the N-terminus, C-
terminus, and/or a side chain of one of the amino acid

10   residues. Multiple vehicles may be used, such as, for
example, Fc domains (Fc) at each terminus.

The Fc domain may be fused to the N or C termini
of the peptide or at both the N and C termini. A
native Fc may be extensively modified to form an Fc

15   analog in accordance with the invention, provided that
binding to the intended substrate (i.e., NGF) is
maintained; see, for example, WO 97/34631 and WO
96/32478. In such Fc variants, one may remove one or
more sites of a native Fc that provide structural

20   features or functional activity not required by the
fusion molecules of the invention. One may remove
these sites by, for example, substituting or deleting
residues, inserting residues into the site, or
truncating portions containing the site. The inserted

25   or substituted residues may also be altered amino
acids, such as peptidomimetics or D-amino acids. Fc
variants may be desirable for a number of reasons, and
several of them are described below. Exemplary Fc
variants include molecules and sequences in which:

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1. Sites involved in disulfide bond formation are
removed. Such removal may avoid reaction with other
cysteine-containing proteins present in the host cell
used to produce the molecules of the invention. For
5   this purpose, the cysteine-containing segment at the N-
terminus may be truncated or cysteine residues may be
deleted or substituted with other amino acids (for
example, alanyl or seryl).

10   2. A native Fc is modified to make it more
compatible with a selected host cell. For example, one
may remove the PA sequence near the N-terminus of a
typical native Fc, which may be recognized by a
digestive enzyme in *E. coli* such as proline
15   iminopeptidase. One may also add an N-terminal
methionine residue, especially when the molecule is
expressed recombinantly in a bacterial cell such as *E.
coli*. The Fc domain of SEQ ID NO: 60 (Figure 2) is one
such Fc variant. Such an Fc variant is preferred for
20   certain embodiments of the present invention, in
particular, those embodiments having the formulas:

$$F_c-P \qquad \qquad (V)$$

wherein the Fc domain is attached to the N-terminus of
the peptide;

25                          $$F_c-(X^2) \qquad \qquad (VI)$$
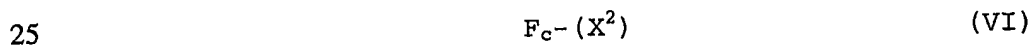
wherein the Fc domain is attached to the N-terminus of
the linker-peptide component (X^2) of a modified
peptide; and most preferably

$$F_c-(L)_c-P \qquad \qquad (VII)$$

30   wherein the Fc domain is attached at the N-terminus of

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the linker-peptide component (X^2) of a modified peptide
.and wherein (X^2) has the formula $-(L)_c-P$.

Embodiments of the present invention also include
modified peptides of the formulas:

5 $P-F_c$ (VIII)

wherein a Fc domain is attached to the C-terminus of
the peptide;

                                 $(X^1)- F_c$ (IX)

wherein the Fc domain is attached to the C-terminus of
10   the peptide-linker component (X^1); and

                             $P-(L)_c-F_c$ (X)

wherein the Fc domain is attached at the C-terminus of
the linker-peptide component (X^2) and wherein (X^2) has
the formula $P-(L)_c$. For modified peptides of the
15   formula (VIII)-(X), the preferred vehicle is a
Fc variant wherein the Fc domain shown in SEQ ID NO:60
lacks the methionine residue shown at position 1 of SEQ
ID NO:60.

20   3. A portion of the N-terminus of a native Fc is
removed to prevent N-terminal heterogeneity when
expressed in a selected host cell. For this purpose,
one may delete any of the first twenty amino acid
residues at the N-terminus, particularly those at
25   positions 1, 2, 3, 4 and 5. Such an Fc variant is
preferred for certain embodiments of the present
invention, in particular, when the vehicle (F^1) is
attached to the N-terminus of the peptide or linker-
peptide component of a modified peptide of the present
30   invention.

4.   One or more glycosylation sites are removed.
Residues that are typically glycosylated (e.g.,
asparagine) may confer cytolytic response.   Such
residues may be deleted or substituted with residues

5   that are not glycosylated (e.g., alanine).

5.   Sites involved in interaction with complement,
such as the C1q binding site, are removed.   For
example, one may delete or substitute the EKK sequence

10 of human IgG1.   Complement recruitment may not be
advantageous for the molecules of the invention   and so
may be avoided with such an Fc variant.

6.   Sites are removed that affect binding to Fc

15 receptors other than a salvage receptor.   A native Fc
may have sites for interaction with certain white blood
cells that are not required for the fusion molecules of
the present invention and so may be removed.

20   7.   The ADCC site is removed.   ADCC sites are
known in the art; see, for example, Molec. Immunol.,
Volume 29 Number 5, pages 633-639 (1992) with regard to
ADCC sites in IgG1.   These sites, as well, are not
required for the modified peptides (fusion products) of

25 the present invention and so may be removed.

8.   When the native Fc is derived from a non-human
antibody, the native Fc may be humanized.   Typically,
to humanize a native Fc, one will substitute selected

30 residues in the non-human native Fc with residues that

40

are normally found in human native Fc. Techniques for
antibody humanization are well known in the art.

As between the peptides or modified peptides of
the present invention and substantial homologs thereof,
5   it is preferable that no more than six residues in the
P region, other than at termini, are different. More
preferably, substantial homologs contemplated by the
present invention include molecules with up to about
six amino acid substitions, insertions, or deletions at
10   any particular locus, other than at a termini, of the P
region of a peptide or modified peptide of the present
invention. Most preferably, the divergence in sequence
between a peptide or modified peptide and a substantial
homolog thereof, particularly in the specified P
15   region, is in the form of "conservative modifications".

Linkers. Any "linker" group is optional. When
present, its chemical structure is not critical, since
it serves primarily as a spacer. The linker is
20   preferably made up of amino acids linked together by
peptide bonds. Thus, in preferred embodiments, the
linker is made up of from 1 to 20 amino acids linked by
peptide bonds, wherein the amino acids are selected
from the twenty naturally occurring amino acids. Some
25   of these amino acids may be glycosylated, as will be
understood by those skilled in the art. In a more
preferred embodiment, the 1 to 20 amino acids are
selected from glycine, alanine, proline, asparagine,
glutamine, and lysine. Even more preferably, a linker
30   is made up of a majority of amino acids that are
sterically unhindered, such as glycine and alanine.

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Thus, preferred linkers are polyglycines, particularly

(Gly)$_4$ (SEQ ID NO: 284), (Gly)$_5$ (SEQ ID NO: 285), (Gly)$_7$

(SEQ ID NO: 286), as well as poly(Gly-Ala) and

polyalanines. Other specific examples of linkers are:

5   (Gly)$_3$Lys(Gly)$_4$ (SEQ ID NO: 123);

                 (Gly)$_3$AsnGlySer(Gly)$_2$ (SEQ ID NO: 124);

                 (Gly)$_3$Cys(Gly)$_4$ (SEQ ID NO: 125); and

                 GlyProAsnGlyGly (SEQ ID NO: 126).

10   To explain the above nomenclature, for example,

(Gly)$_3$Lys(Gly)$_4$ means Gly-Gly-Gly-Lys-Gly-Gly-Gly-Gly.

Combinations of Gly and Ala are also preferred. The

linkers shown here are merely exemplary; linkers within

the scope of the invention may be much longer and may

15 include other residues.

         Non-peptide linkers are also possible. For

example, alkyl linkers such as -NH-(CH$_2$)$_s$-C(O)-,

wherein s = 2-20 could be used. These alkyl linkers

may further be substituted by any non-sterically

20 hindering group such as lower alkyl (e.g., C$_1$-C$_6$) lower

acyl, halogen (e.g., Cl, Br), CN, NH$_2$, phenyl, etc. An

exemplary non-peptide linker is a PEG linker,

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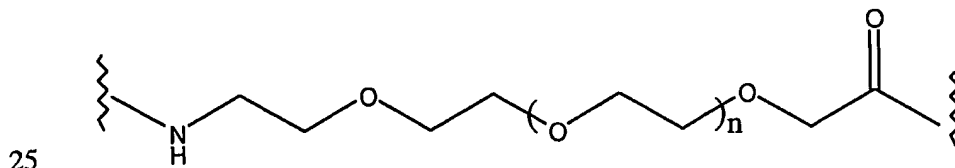
wherein n is such that the linker has a molecular

weight of 100 to 5000 kilodaltons (kD), preferably 100

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to 500 kD. The peptide linkers may be altered to form derivatives in the same manner as described above.

<u>Derivatives</u>. Also contemplated are derivatives of the
5   peptides or the modified peptides of the present invention. Such derivatives may improve the solubility, absorption, biological half-life, and the like, of the peptides or modified peptides. The moieties may alternatively eliminate or attenuate any
10   undesirable side-effect of the peptides or modified peptides, and the like. Exemplary derivatives include peptides or modified peptides in which:

    1.   The peptide or modified peptide or some
15   portion thereof is cyclic. For example, the peptide or peptide portion of a modified peptide may be modified to contain two or more Cys residues (e.g., in the linker), which could cyclize by disulfide bond formation. For citations to references on the
20   preparation of cyclized derivatives, see WO 00/24782.

    2.   The peptide or modified peptide is cross-linked or is rendered capable of cross-linking between molecules. For example, the peptide or peptide portion
25   of a modified peptide may be modified to contain one Cys residue and thereby be able to form an intermolecular disulfide bond with a like molecule. The modified peptide may also be cross-linked through its C-terminus, as in the molecule shown below.
30

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$$F^1-(X^1)_b-CO-\overset{H}{N}\diagdown\diagdown\diagdown\diagdown\overset{O}{\underset{}{\overset{||}{C}}}NH_2$$

$$F^1-(X^1)_b-CO-\overset{H}{N}\diagdown\diagdown\underset{\overset{||}{O}}{C}NH$$
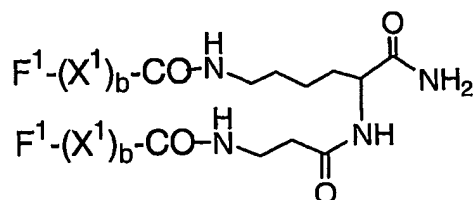
 3.   One or more peptidyl [-C(O)NR-] linkages
(bonds) is replaced by a non-peptidyl linkage.

5   Exemplary non-peptidyl linkages are -CH$_2$-carbamate
[-CH$_2$-OC(O)NR-], phosphonate, -CH$_2$-sulfonamide [-CH$_2$-
S(O)$_2$NR-], urea [-NHC(O)NH-], -CH$_2$-secondary amine, and
alkylated peptide [-C(O)NR^6- wherein R^6 is lower
alkyl].

10

 4.   The N-terminus is derivatized. Typically, the
N-terminus may be acylated or modified to a substituted
amine. Exemplary N-terminal derivative groups include
-NRR^1 (other than -NH$_2$), -NRC(O)R^1, -NRC(O)OR^1,

15   -NRS(O)$_2$R^1, -NHC(O)NHR^1, succinimide, or
benzyloxycarbonyl-NH- (CBZ-NH-), wherein R and R^1 are
each independently hydrogen or lower alkyl and wherein
the phenyl ring may be substituted with 1 to 3
substituents selected from the group consisting of C$_1$-

20   C$_4$ alkyl, C$_1$-C$_4$ alkoxy, chloro, and bromo.

 5.   The free C-terminus is derivatized.
Typically, the C-terminus is esterified or amidated.
For example, one may use methods described in the art

25   to add (NH-CH$_2$-CH$_2$-NH$_2$)$_2$ to modified peptides of the
invention having any of SEQ ID NOS: 1 to 58 at the C-
terminus. Likewise, one may use methods described in
the art to add -NH$_2$ to modified peptides of the

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invention having any of SEQ ID NOS: 1 to 58 at the C-
terminus. Exemplary C-terminal derivative groups
include, for example, -C(O)R^2 wherein R^2 is lower
alkoxy or -NR^{3}R^4 wherein R^3 and R^4 are independently
5   hydrogen or C$_1$-C$_8$ alkyl (preferably C$_1$-C$_4$ alkyl).

     6. A disulfide bond is replaced with another,
preferably more stable, cross-linking moiety (e.g., an
alkylene). See, for example, Bhatnagar et al., J. Med.
10   Chem., Volume 39, pages 3814-3819 (1996); Alberts et
al., Thirteenth Am. Pep. Symp., pages 357-359 (1993).

     Derivatization with bifunctional agents is useful for
cross-linking the peptides or modified peptides or their
15   functional derivatives to a water-insoluble support matrix
or to other macromolecular vehicles. Commonly used cross-
linking agents include, e.g., 1,1-bis(diazoacetyl)-2-
phenylethane, glutaraldehyde, N-hydroxysuccinimide esters,
for example, esters with 4-azidosalicylic acid,
20   homobifunctional imidoesters, including disuccinimidyl
esters such as 3,3'-dithiobis(succinimidylpropionate), and
bifunctional maleimides such as bis-N-maleimido-1,8-
octane. Derivatizing agents such as methyl-3-[(p-
azidophenyl)dithio]-propioimidate yield photo-activatable
25   intermediates that are capable of forming crosslinks in
the presence of light. Alternatively, reactive water-
insoluble matrices such as cyanogen bromide-activated
carbohydrates and the reactive substrates described in
U.S. Patent Nos. 3,969,287; 3,691,016; 4,195,128;
30   4,247,642; 4,229,537; and 4,330,440 are employed for
protein immobilization.

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Carbohydrate (oligosaccharide) groups may conveniently be attached to sites that are known to be glycosylation sites in proteins. Generally, O-linked oligosaccharides are attached to serine (Ser) or
5   threonine (Thr) residues, while N-linked oligo-saccharides are attached to asparagine (Asn) residues when they are part of the sequence Asn-X-Ser/Thr, where X can be any amino acid except proline. X is preferably one of the nineteen naturally occurring
10 amino acids other than proline. The structures of N-linked and O-linked oligosaccharides and the sugar residues found in each type are different. One type of sugar that is commonly found on both is N-acetylneuraminic acid (referred to as sialic acid).
15 Sialic acid is usually the terminal residue of both N-linked and O-linked oligosaccharides and, by virtue of its negative charge, may confer acidic properties to the glycosylated modified peptide. Such site(s) may be incorporated in the linker of the modified peptides of
20 the invention and are preferably glycosylated by a cell during recombinant production of the polypeptide modified peptides (e.g., in mammalian cells such as CHO, BHK, COS). However, such sites may further be glycosylated by synthetic or semi-synthetic procedures
25 known in the art.

Other possible modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, oxidation of the sulfur atom in Cys, methylation of the alpha-amino
30 groups of lysine, arginine, and histidine side chains.

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Creighton, Proteins: Structure and Molecule Properties,

W. H. Freeman & Co., San Francisco, pages 79-86 (1983).

Also contemplated are the chemical modifications

of the peptides by the attachment of at least one

5   moiety wherein said moiety permits an increase in

overall stability of the modified peptide and increase

in circulation time in the body. Moieties useful as

covalently attached vehicles in the invention may also

be used for this purpose. Examples of such moieties

10   include: polyethylene glycol (PEG), copolymers of

ethylene glycol and propylene glycol, carboxymethyl

cellulose, dextran, polyvinyl alcohol, polyvinyl

pyrrolidone and polyproline. See, for example,

Abuchowski and Davis, Soluble Polymer-Enzyme Adducts,

15   Enzymes as Drugs, Hocenberg and Roberts, eds., Wiley-

Interscience, New York, NY, pages 367-383 (1981);

Newmark et al., J. Appl. Biochem. Volume 4, pages 185-

189 (1982). Other polymers that could be used are

poly-1,3-dioxolane and poly-1,3,6-trioxocane.

20   Preferred are PEG moieties.

Peptides and modified peptides of the present

invention may be changed at the DNA level, as well. The

DNA sequence of any portion of the modified peptide may

be changed to codons more compatible with the chosen

25   host cell. For *E. coli*, which is the preferred host

cell, optimized codons are known in the art. Codons

may be substituted to eliminate restriction sites or to

include silent restriction sites, which may aid in

processing of the DNA in the selected host cell. The

30   vehicle, linker and peptide DNA sequences may be

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modified to include any of the foregoing sequence
changes.

Methods of Making

5   The modified peptides of the invention, for the
most part, may be made in transformed host cells using
recombinant DNA techniques. To do so, a recombinant
DNA molecule coding for the peptide is prepared.
Methods of preparing such DNA molecules are well known
10   in the art. Reference works on the general principles
of recombinant DNA Technology include Watson et al.,
Molecular Biology of the Gene, Volumes I and II, The
Benjamin/Cummings Publishing Company, Inc., Menlo Park,
Calif. (1987); Darnell et al., *Molecular Cell Biology*,
15   Scientific American Books, Inc., New York, N.Y. (1986);
Lewin, *Genes II*, John Wiley & Sons, New York, N.Y.
(1985); Old, et al., *Principles of Gene Manipulation:
An Introduction to Genetic Engineering*, 2k edition,
University of California Press, Berkeley, Calif.
20   (1981); Sambrook et al., *Molecular Cloning, A
Laboratory Manual*, Cold Spring Harbor Laboratory Press,
Cold Spring Harbor, NY (1989); and Ausubel et al,
Current Protocols in Molecular Biology, Wiley
Interscience, N.Y., (1987, 1992). These references are
25   herein entirely incorporated by reference as are the
references cited therein.
       For instance, sequences coding for the peptides
can be excised from DNA using suitable restriction
enzymes. Alternatively, the DNA molecule can be
30   synthesized using chemical synthesis techniques, such

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as the phosphoramidate method.   Also, a combination of
these techniques can be used.

The invention also includes a vector capable of
expressing the peptides or modified peptides in an
5   appropriate host.   The vector comprises the DNA
molecule that codes for the peptides or modified
peptides operatively linked to appropriate expression
control sequences.   Methods of effecting this operative
linking, either before or after the DNA molecule is
10   inserted into the vector, are well known.   Expression
control sequences include promoters, activators,
enhancers, operators, ribosomal binding sites, start
signals, stop signals, cap signals, polyadenylation
signals, and other signals involved with the control of
15   transcription or translation.

The resulting vector having the DNA molecule
thereon is used to transform an appropriate host.
This transformation may be performed using methods well
known in the art.

20   Any of a large number of available and well-known
host cells may be used in the practice of the
invention.   The selection of a particular host is
dependent upon a number of factors recognized by the
art.   These include, for example, compatibility with
25   the chosen expression vector, toxicity of the peptides
encoded by the DNA molecule, rate of transformation,
ease of recovery of the peptides or modified peptides,
expression characteristics, bio-safety, and costs.   A
balance of these factors must be struck, with the
30   understanding that not all hosts may be equally

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effective for the expression of a particular DNA
sequence.

Within these general guidelines, useful microbial
hosts include bacteria from the genera Bacillus,
5   Escherichia (such as *E. coli*), Pseudomonas,
Streptomyces, Salmonella, Erwinia, and yeasts from the
genera Hansenula, Kluyveromyces, Pichia, Rhino-
sporidium, Saccharomyces, and Schizosaccharomyces, and
other fungi.   The more preferred hosts are
10 microorganisms of the species *Pichia pastoris, Bacillus
subtilis, Bacillus brevis, Saccharomyces cerevisiae,*
the various strains of *Escherichia coli* (*e.g.*, HB101,
(ATCC NO. 33694) DH5α, DH10, and MC1061 (ATCC NO.
53338)), *and Yarrowia lipolytica*.

15   A number of suitable mammalian host cells are also
known in the art and many are available from the
American Type Culture Collection (ATCC), 10801
University Boulevard, Manassas, VA 20110-2209.
Examples include, but are not limited to, mammalian
20 cells, such as Chinese hamster ovary cells (CHO) (ATCC
No. CCL61) CHO DHFR-cells (Urlaub *et al., Proc. Natl.
Acad. Sci. USA*, 97:4216-4220 (1980)), human embryonic
kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), or
3T3 cells (ATCC No. CCL92).   The selection of suitable
25 mammalian host cells and methods for transformation,
culture, amplification, screening and product
production and purification are known in the art.
Other suitable mammalian cell lines, are the monkey
COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No.
30 CRL1651), and the CV-1 cell line (ATCC No. CCL70).
Further exemplary mammalian host cells include primate

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cell lines and rodent cell lines, including transformed

cell lines. Normal diploid cells, cell strains derived

from *in vitro* culture of primary tissue, as well as

primary explants, are also suitable. Candidate cells

5   may be genotypically deficient in the selection gene,

or may contain a dominantly acting selection gene.

Other suitable mammalian cell lines include, but are

not limited to, mouse neuroblastoma N2A cells, HeLa,

mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c

10   or NIH mice, BHK or HaK hamster cell lines, which are

available from the ATCC. Each of these cell lines is

known by and available to those skilled in the art of

protein expression.

Many strains of yeast cells known to those skilled

15   in the art are also available as host cells for the

expression of the polypeptides of the present

invention. Preferred yeast cells include, for example,

saccharomyces cerivisae and *pichia pastoris*.

Additionally, where desired, insect cell systems

20   may be utilized in the methods of the present

invention. Such systems are described for example in

Kitts *et al.*, *Biotechniques*, 14:810-817 (1993);

Lucklow, *Curr. Opin. Biotechnol.*, 4:564-572 (1993); and

Lucklow *et al.* (*J. Virol.*, 67:4566-4579 (1993).

25   Preferred insect cells are Sf-9 and HI5 (Invitrogen,

Carlsbad, Ca). One may also use transgenic animals to

express the peptides and modified peptides of the

present invention. For example, one may use a

transgenic milk-producing animal (a cow or goat, for

30   example) and obtain the peptide or modified peptide in

the animal milk. One may also use plants to produce

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the peptides and modified peptides of the present
invention, however, in general, the glycosylation
occurring in plants is different from that produced in
mammalian cells, and may result in a glycosylated
5   product which is not suitable for human therapeutic
use.

The transformed host is cultured and a single
clonal isolate is purified. Host cells may be cultured
under conventional fermentation conditions so that the
10   desired modified peptides are expressed. Such
fermentation conditions are well known in the art. Any
promoter which is functional in the host cell may be
used to control gene expression.

Preferably the modified peptides, or at least the
15   peptide portion thereof, of the invention are secreted.
If the modified peptide or peptide portion thereof is
secreted, the peptides can be purified from culture by
methods well known in the art.

If the modified peptide or peptide portion thereof
20   is expressed in bacterial hosts as insoluble inclusion
bodies the modified peptides or peptide portion thereof
can be harvested from host cells in accordance with
methods known in the art. For example, the
solubilization of washed and frozen inclusion bodies
25   can be accomplished by the addition of a buffer
containing a chaotropic agent and a reducing agent to
thawed inclusion bodies. Preferably, the
solubilization mixture is diluted into the refold
buffer to form the correct protein conformation and
30   disulfide bonds. Redox reagents may be added to the
refold buffer just prior to the addition of the

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solubilization mixture.   The refold solution may be
passed through a filter system to remove particulate
matter and then be concentrated by low temperature
(approximately 5ºC) ultrafiltration (UF).   Low
5   temperature dialfiltration (DF) may be performed also
to remove low molecular weight solutes.   Precipitation
and clarification at an acidic pH is generally carried
out to remove the majority of host cell impurities,
product aggregates, and misfolded impurities.   Residual
10   host cell impurities and product aggregates may be
removed by cation exchange chromatography e.g., on SP
Sepharose FF media, Q Sepharose HP column, and/or
Ceramic Hydroxyapatite resin (Bio-Rad, Hercules, CA).

The modified peptides, or at least the peptide
15   portion thereof, may also be made by synthetic methods.
For example, solid phase synthesis techniques may be
used.   Suitable techniques are well known in the art,
and include those described in Merrifield, Chem.
Polypeptides, pages 335-361 (Katsoyannis and Panayotis
20   editors) (1973); Merrifield, J. Am. Chem. Soc., Volume
85, page 2149 (1963); Davis et al., Biochem. Intl.,
Volume 10, pages 394-414 (1985); Stewart and Young,
Solid Phase Peptide Synthesis (1969); U.S. Patent No.
3,941,763; Finn et al., The Proteins (3d edition),
25   Volume 2, pages 105-253 (1976); and Erickson et al.,
The Proteins (Third Edition), Volume 2, pages 257-527
(1976).   Solid phase synthesis is the preferred
technique for making individual peptides because of its
cost-effectiveness.

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Modified peptides that contain non-peptide groups may be synthesized by well-known organic chemistry techniques.

5   Pharmaceutical Compositions

In General. The present invention also provides methods of using pharmaceutical compositions of the inventive peptides and/or modified peptides, e.g., in the
10   prevention or treatment of pain (including, but not limited to, inflammatory pain and associated hyperalgesia and allodynia, neuropathic pain and associated hyperalgesia and allodynia, diabetic neuropathy pain, causalgia, sympathetically maintained pain,
15   deafferentation syndromes, acute pain, tension headache, migraine, dental pain, pain from trauma, surgical pain, pain resulting from amputation or abscess, causalgia, demyelinating diseases, and trigeminal neuralgia). The peptides and modified peptides of the invention have
20   therapeutic value for the prevention or treatment of other diseases linked to NGF as a causative agent, including, but not limited to, asthma, urge incontinence (i.e., hyperactive bladder), psoriasis, cancer (especially, pancreatic cancer and melanoma), chronic alcoholism,
25   stroke, thalamic pain syndrome, diabetes, acquired immune deficiency syndrome ("AIDS"), toxins and chemotherapy, general headache, migraine, cluster headache, mixed-vascular and non-vascular syndromes, general inflammation, arthritis, rheumatic diseases, lupus, osteoarthritis,
30   inflammatory bowel disorders, inflammatory eye disorders, inflammatory or unstable bladder disorders, psoriasis,

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skin complaints with inflammatory components, sunburn,
carditis, dermatitis, myositis, neuritis, collagen
vascular diseases, chronic inflammatory conditions,
asthma, epithelial tissue damage or dysfunction, herpes
5   simplex, disturbances of visceral motility at respiratory,
genitourinary, gastrointestinal or vascular regions,
wounds, burns, allergic skin reactions, pruritis,
vitiligo, general gastrointestinal disorders, colitis,
gastric ulceration, duodenal ulcers, vasomotor or allergic
10 rhinitis, or bronchial disorders.

The invention also provides for the use of the
peptides and/or modified peptides of the present
invention for the prevention or treatment of the same
diseases listed above.

15   Accordingly, the present invention also relates to
the use of one or more of the peptide and/or modified
peptides of the present invention in the manufacture of a
medicament for the treatment of a disorder such as any one
of those mentioned above.

20   Such pharmaceutical compositions or medicaments may
be for administration by injection, or for oral,
pulmonary, nasal, transdermal or other forms of
administration. In general, the invention encompasses
pharmaceutical compositions comprising effective amounts
25 of a peptide or modified peptide of the invention (in
amounts effective to prevent, ameliorate, or abolish pain
or any of the other medical conditions provided herein)
together with pharmaceutically acceptable diluents,
preservatives, solubilizers, emulsifiers, adjuvants and/or
30 carriers. Such compositions include diluents of various
buffer content (e.g., Tris-HCl, acetate, phosphate), pH

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and ionic strength; additives such as detergents and
solubilizing agents (e.g., Tween 80, Polysorbate 80),
anti-oxidants (e.g., ascorbic acid, sodium
metabisulfite), preservatives (e.g., Thimerosol, benzyl
5 alcohol) and bulking substances (e.g., lactose, mannitol);
incorporation of the material into particulate
preparations of polymeric modified peptides such as
polylactic acid, polyglycolic acid, etc. or into
liposomes. Hyaluronic acid may also be used, and this may
10 have the effect of promoting sustained duration in the
circulation. Such compositions may influence the physical
state, stability, rate of *in vivo* release, and rate of *in
vivo* clearance of the present proteins and derivatives.
See, for example, Remington's Pharmaceutical Sciences,
15 18th Edition., Mack Publishing Co., Easton, PA, pages
1435-1712 (1990), which is herein incorporated by
reference. The compositions may be prepared in liquid
form, or as a dried powder (such as lyophilized form).
Implantable sustained release formulations are also
20 contemplated, as are transdermal formulations.

Oral dosage forms. Contemplated for use herein
are oral solid dosage forms, which are described
generally in Chapter 89 of Remington's Pharmaceutical
25 Sciences, above, which is herein incorporated by
reference. Solid dosage forms include tablets,
capsules, pills, troches or lozenges, cachets or
pellets. Also, liposomal or proteinoid encapsulation
may be used to formulate the present compositions (such
30 as, for example, the proteinoid microspheres reported
in U.S. Patent No. 4,925,673). Liposomal encapsulation

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may be used, and the liposomes may be derivatized with
various polymers (see, for example, U.S. Patent No.
5,013,556). A description of possible solid dosage
forms is given in Chapter 10 of Marshall, K., Modern
5   Pharmaceutics, edited by G. S. Banker and C. T. Rhodes
(1979), herein incorporated by reference. In general,
the formulation will include a modified peptide of the
invention , as well as inert ingredients which allow
for protection against the stomach environment and
10   release of the modified peptide in the intestine.

Also specifically contemplated are oral dosage
forms of the inventive peptides or modified peptides
themselves. In this regard, if necessary, the peptides
or modified peptides may be chemically modified so that
15   oral delivery is efficacious. It is also possible to
use a salt of a modified aliphatic amino acid, such as
sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC),
as a carrier to enhance absorption of the modified
peptides of the invention. See U.S. Patent No.
20   5,792,451, entitled "Oral Drug Delivery Composition and
Methods".

The peptides or modified peptides of the invention
can be included in the formulation as fine
multiparticulates in the form of granules or pellets of
25   a particle size about one millimeter. The formulation
of the material for capsule administration could also
be as a powder, as lightly compressed plugs, or even as
tablets. The therapeutic could be prepared by
compression.

30   Colorants and flavoring agents may all be
included. For example, the peptide or modified peptide

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or derivative thereof may be formulated (such as by
liposome or microsphere encapsulation) and then further
contained within an edible product, such as a
refrigerated beverage containing colorants and
5   flavoring agents.

One may dilute or increase the volume of the
peptide or modified peptide of the invention with an
inert material. These diluents could include
carbohydrates, especially, mannitol, α-lactose,
10   anhydrous lactose, cellulose, sucrose, modified
dextrans and starch. Certain inorganic salts may also
be used as fillers, including calcium triphosphate,
magnesium carbonate and sodium chloride. Some
commercially available diluents are Fast-Flo, Emdex,
15   STA-Rx 1500, Emcompress and Avicell.

Disintegrants may be included in the formulation
of the therapeutic into a solid dosage form. Materials
used as disintegrants include, but are not limited to,
starch, including the commercially available
20   disintegrant based on starch, Explotab. Sodium starch
glycolate, Amberlite, sodium carboxymethylcellulose,
ultramylopectin, sodium alginate, gelatin, orange peel,
acid carboxymethyl cellulose, natural sponge and
bentonite may also be used. Another form of the
25   disintegrants are the insoluble cationic exchange
resins. Powdered gums may be used as disintegrants and
as binders, and these can include powdered gums such as
agar, Karaya or tragacanth. Alginic acid and its
sodium salt are also useful as disintegrants.
30   Binders may be used to hold the components of the
pharmaceutical composition together to form a hard

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tablet, and they include materials from natural
products such as acacia, tragacanth, starch and
gelatin.   Others include methyl cellulose (MC), ethyl
cellulose (EC) and carboxymethyl cellulose (CMC).

5   Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl
cellulose (HPMC) could both be used in alcoholic
solutions to granulate the therapeutic.

An anti-frictional agent may be included in the
formulation to prevent sticking during the formulating

10   process.   Lubricants may be used as a layer between the
therapeutic and the die wall, and these can include,
but are not limited to: stearic acid, including its
magnesium and calcium salts, polytetrafluoroethylene
(PTFE), liquid paraffin, vegetable oils and waxes.

15   Soluble lubricants may also be used such as sodium
lauryl sulfate, magnesium lauryl sulfate, polyethylene
glycol of various molecular weights, Carbowax 4000 and
6000.

Glidants that might improve the flow properties of

20   the modified peptide during formulation and to aid
rearrangement during compression might be added.   Such
glidants may include starch, talc, pyrogenic silica and
hydrated silicoaluminate.

To aid dissolution of the peptide or modified

25   peptide of the invention into the aqueous environment,
a surfactant might be added as a wetting agent.   Such
surfactants may include anionic detergents such as
sodium lauryl sulfate, dioctyl sodium sulfosuccinate
and dioctyl sodium sulfonate.   Cationic detergents may

30   be used and can include benzalkonium chloride or
benzethonium chloride.   The list of potential nonionic

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detergents that may be included in the formulation as
surfactants are lauromacrogol 400, polyoxyl 40
stearate, polyoxyethylene hydrogenated castor oil 10,
50 and 60, glycerol monostearate, polysorbate 40, 60,
5   65 and 80, sucrose fatty acid ester, methyl cellulose
and carboxymethyl cellulose. These surfactants may be
present in the formulation either alone or as a mixture
in different ratios.

     Additives may also be included in the formulation
10   to enhance uptake of the peptide or modified peptide.
Additives potentially having this property include
various fatty acids, such as, for instance, oleic acid,
linoleic acid and linolenic acid.

     Controlled release formulation may be desirable.
15   The peptide or modified peptide of the invention may be
incorporated into an inert matrix which permits release
by either diffusion or leaching mechanisms, for
example, gums. Slowly degenerating matrices may also
be incorporated into the formulation, for example,
20   alginates or polysaccharides. Another form of a
controlled release of the peptide or modified peptide
of the invention is by a method based on the Oros
therapeutic system (Alza Corp.), i.e., the drug is
enclosed in a semipermeable membrane which allows water
25   to enter and push drug out through a single small
opening due to osmotic effects. Some enteric coatings
also have a delayed release effect.

     Other coatings may be used for the formulation.
These include a variety of sugars which could be
30   applied in a coating pan. The therapeutic agent could
also be given in a film-coated tablet, and the

60

materials used in this instance are divided into two
groups. The first are the nonenteric materials and
include methyl cellulose, ethyl cellulose, hydroxyethyl
cellulose, methylhydroxy-ethyl cellulose, hydroxypropyl
5   cellulose, hydroxypropyl-methyl cellulose, sodium
carboxy-methyl cellulose, providone and the
polyethylene glycols. The second group consists of
enteric materials that are commonly esters of phthalic
acid.
10   A mixture of materials may be used to provide the
optimum film coating. Film coating may be carried out
in a pan coater, in a fluidized bed, or by compression
coating.

15   <u>Pulmonary delivery forms</u>. Also contemplated
herein is pulmonary delivery of a pharmaceutical
composition in accordance with the invention. The
peptide or modified peptide (or derivatives thereof) is
delivered to the lungs of a mammal while inhaling and
20   traverses across the lung epithelial lining to the
blood stream. Reports relating to the pulmonary
delivery of macromolecules that may be helpful in this
regard include Adjei et al., Pharma. Res., Volume 7,
pages 565-569 (1990); Adjei et al., Internatl. J.
25   Pharmaceutics, Volume 63, pages 135-144 (1990)
(leuprolide acetate); Braquet et al., J. Cardiovasc.
Pharmacol., Volume 13 (suppl.5), s.143-146 (1989)
(endothelin-1); Hubbard et al., Annals Int. Med.,
Volume 3, pages 206-12 (1989) (α1-antitrypsin); Smith
30   et al., J. Clin. Invest., Volume 84, pages 1145-1146
(1989) (α1-proteinase); Oswein et al., "Aerosolization

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of Proteins", Proc. Symp. Resp. Drug Delivery II,

Keystone, Colorado (1990) (recombinant human growth

hormone); Debs et al., J. Immunol., Volume 140, pages

3482-3488 (1988) (interferon-γ and tumor necrosis

5   factor α); and U.S. Patent No. 5,284,656 (granulocyte

colony stimulating factor).

Contemplated for use in the practice of the

invention are a wide range of mechanical devices

designed for the pulmonary delivery of therapeutic

10 products, including but not limited to nebulizers,

metered dose inhalers, and powder inhalers, all of

which are familiar to those skilled in the art. Some

specific examples of commercially available devices

suitable for the practice of the invention are the

15 Ultravent nebulizer, manufactured by Mallinckrodt,

Inc., St. Louis, Missouri; the Acorn II nebulizer,

manufactured by Marquest Medical Products, Englewood,

Colorado; the Ventolin metered dose inhaler,

manufactured by Glaxo Inc., Research Triangle Park,

20 North Carolina; and the Spinhaler powder inhaler,

manufactured by Fisons Corp., Bedford, Massachusetts.

All such devices require the use of formulations

suitable for the dispensing of the described peptides

and modified peptides. Typically, each formulation

25 is specific to the type of device employed and may

involve the use of an appropriate propellant

material, in addition to diluents, adjuvants and/or

carriers useful in therapy.

The peptides or modified peptides of the

30 invention will most advantageously be prepared in

particulate form, with an average particle size of

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less than 10 micrometers (μm), or microns, and most

preferably in the range from 0.5 to 5 μm, for most

effective delivery to the distal lung.

     Pharmaceutically acceptable carriers for these

5   pulmonary compositions include carbohydrates such as

trehalose, mannitol, xylitol, sucrose, lactose, and

sorbitol. Other ingredients for use in formulations

may include DPPC, DOPE, DSPC and DOPC. Natural or

synthetic surfactants may be used. PEG may be used

10   (even apart from its use in derivatizing the protein

or analog). Dextrans, such as cyclodextran, bile

salts, cellulose and cellulose derivatives may also

be used. Amino acids may be used, such as in a

buffer formulation.

15   In addition, the use of liposomes, microcapsules

or microspheres, inclusion complexes, or other types of

carriers is contemplated.

     Formulations suitable for use with a nebulizer,

either jet or ultrasonic type, will typically comprise

20   the described modified peptide dissolved in water at a

concentration of about 0.1 to 25 milligrams (mg) of

biologically active protein per milliliter (ml) of

solution. The formulation may also include a buffer

and a simple sugar (e.g., for peptide stabilization and

25   regulation of osmotic pressure). The nebulizer

formulation may also contain a surfactant, to reduce or

prevent surface induced aggregation of the protein

caused by atomization of the solution in forming the

aerosol.

30   Formulations for use with a metered-dose inhaler

device will generally comprise a finely divided powder

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containing the described modified peptide suspended in
a propellant with the aid of a surfactant. The
propellant may be any conventional material employed
for this purpose, such as a chlorofluorocarbon, a

5   hydrochlorofluorocarbon, a hydrofluorocarbon, or a
hydrocarbon, including trichlorofluoromethane,
dichlorodifluoromethane, dichlorotetrafluoroethanol,
and 1,1,1,2-tetrafluoroethane, or combinations thereof.
Suitable surfactants include sorbitan trioleate and

10   soya lecithin. Oleic acid may also be useful as a
surfactant.

Formulations for dispensing from a powder inhaler
device will comprise a finely divided dry powder
containing the described modified peptide and may also

15   include a bulking agent, such as lactose, sorbitol,
sucrose, mannitol, trehalose, or xylitol in amounts
which facilitate dispersal of the powder from the
device, e.g., 50 to 90% by weight of the formulation.

20   Nasal delivery forms. Nasal delivery of the
peptides and modified peptides is also contemplated.
Nasal delivery allows the passage of the modified
peptides of the invention to the blood stream directly
after administering the therapeutic product to the

25   nose, without the necessity for deposition of the
product in the lung. Formulations for nasal delivery
include those with dextran or cyclodextran. Delivery
via transport across other mucous membranes is also
contemplated.

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Dosages. The dosage regimen involved in a method for
treating the involved disease or disorder will be
determined by the attending physician, considering various
factors which modify the action of therapeutic agents,

5   such as the age, condition, body weight, sex and diet of
the patient, the severity of the condition being treated,
time of administration, and other clinical factors.
Generally, the daily regimen should be in the range of
1.0-10000 micrograms (µg) of the modified peptide per

10   kilogram (kg) of body weight, preferably 1.0-1000 µg per
kilogram of body weight, and most preferably 1.0-150 µg
per kilogram of body weight.

EXAMPLES

15

The peptides and modified peptides of the
invention may be prepared as described below. These
examples comprise preferred embodiments of the
invention and are intended to be illustrative only and

20   not limiting.

**Example 1: Identification of NGF-Inhibitory Peptides
by Peptide Phage Display**

25 1.   NGF-coated magnetic bead preparation

A.   Biotinylation of NGF protein. Human
recombinant NGF was biotinylated using the EZ-link
Sulfo-NHS-LC-Biotinylation Kit (Pierce, Rockford, IL)

30   according to the manufacturer's suggestions. The
biotinylated NGF protein was dialyzed in phosphate

65

buffered saline (PBS) to remove any free-floating
biotin from the solution. The biotinylated NGF protein
solution was passed through the Immunopure Immobilized
Monomeric Avidin column (Pierce, Rockford, IL) to
5   further remove any unbound or loosely bound biotins.
The biotinylated NGF protein-containing solution was
concentrated using Centricon units (Amicon, Bedford,
MA), and the final protein concentration was determined
using Bio-Rad Protein Assay reagent (Bio-Rad Labs,
10   Hercules, CA). The purified and concentrated
biotinylated NGF was shown to be fully active in the
DRG neuron-based NGF neutralization bioassay (see
further below in Example 3) and also in an SCG neuron-
based neutralization assay (not described here).
15

        B.   NGF immobilization on magnetic beads. The
biotinylated NGF protein was immobilized on
Streptavidin Dynabeads (Dynal, Lake Success, NY) at a
concentration of 2 milligrams (mg) of biotinylated NGF
20   protein per 100 milliters (ml) of the bead stock from
the manufacturer. By drawing the beads to one side of
a tube using a magnet and pipetting away the liquid,
the beads were washed twice with phosphate buffered
saline (PBS) and resuspended in PBS. Biotinylated NGF
25   protein was added to the washed beads at the above
concentration and incubated, with rotation, for one
hour at room temperature. NGF-coated beads were then
blocked by adding BSA to 1% final concentration and
incubating overnight at 4°C, with rotation. The
30   resulting NGF-coated beads were then washed five times

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with PBST (i.e., PBS with 0.05% Tween-20) before being
subjected to the selection procedures.

        C.   Negative selection bead preparation.
5   Additional beads were also prepared for negative
    selections. For each panning condition, 250
    microliters (µl) of the bead stock from the
    manufacturer was subjected to the above procedure
    (Section 1.A., above) except that the incubation step
10 with biotinylated NGF was omitted. In the last washing
    step, the beads were divided into five 50-ml aliquots.

    2.   Selection of NGF binding phage

15   A.   Overall strategy. Three filamentous phage
    libraries, TN8-IX (5 X 10^9 independent transformants),
    TN12-I (1.4 X 10^9 independent transformants), and
    Linear (2.3 X 10^9 independent transformants) (Dyax
    Corp., Cambridge, MA), were used to select for NGF
20 binding phage. Each library was subjected to antibody
    elution (Section 2.D.), receptor elution (Section
    2.E.), and bead elution (Section 2.F.). Thus, nine
    different panning conditions were carried out (TN8-IX
    using the antibody elution method, TN8-IX using the
25 receptor elution method, TN8-IX using the bead elution
    method, TN12-I using the antibody elution method, TN12-
    I using the receptor elution method, and TN12-I using
    the bead elution method, Linear using the antibody
    elution method, Linear using the receptor elution
30 method, and Linear using the bead elution method).

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Three rounds of selection were performed for each
condition.

    B.   Negative selection. For each panning
5   condition, about 100 random library equivalent for TN8-
IX and TN12-I libraries (5 X 10^{11} pfu for TN8-IX, and
1.4 X 10^{11} pfu for TN12-I) and about 10 random library
equivalent for Linear library (2.3 X 10^{10} pfu) were
aliquoted from the library stock and diluted to 300 µl
10 of PBST. After the last washing liquid was drawn out
from the first 50-µl aliquot of the beads prepared for
negative selections (Section 1.B.), the 300 µl-diluted
library stock was added to the beads. The resulting
mixture was incubated for ten minutes at room
15 temperature with rotation. The phage supernatant was
drawn out using the magnet and added to the second 50
µl aliquot for another negative selection step. In
this manner, five negative selection steps were
performed.
20

    C.   Selection using the NGF protein coated beads.
The phage supernatant after the last negative selection
step (Section 1.B., above) was added to the NGF-coated
beads after the last washing step (Section 1.A.,
25 above). This mixture was incubated with rotation for
one to two hours at room temperature, allowing specific
phage to bind to the target protein. After the
supernatant was discarded, the beads were washed seven
times with PBST.
30

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D.   <u>Antibody elution of bound phage</u>.   After the
last washing step (Section 2.C., above), the bound
phages were eluted from the magnetic beads by adding
100 μl of 10 μM Monoclonal Anti-Human Nerve Growth

5   Factor-β Clone 25623.1 (Catalog No. N-3279, Sigma, St.
Louis, MO).   After one hour of incubation with rotation
at room temperature, the liquid containing the eluted
phage was drawn out and transferred to another tube.
Nine hundred microliters of Min A Salts solution (60 mM

10   K_2HPO_4, 33 mM KH_2PO_4, 7.6 mM $(NH_4)SO_4$, and 1.7 mM sodium
citrate) were added to bring the final volume to 1 ml.

E.   <u>Receptor (trkA) elution of bound phage</u>.
After the last washing step (Section 2.C., above), the

15   bound phages were eluted from the magnetic beads by
adding 100 μl of 100 μM soluble NGF receptor, trkA (Ig-
like extracellular subdomain; amino acids 280-384).
After one hour of incubation with rotation at room
temperature, the liquid containing the eluted phage was

20   drawn out and transferred to another tube.   Nine
hundred microliters of Min A Salts solution (60 mM
K_2HPO_4, 33 mM KH_2PO_4, 7.6 mM $(NH_4)SO_4$, and 1.7 mM sodium
citrate) were added to make the final volume to 1 ml.

25   F.   <u>Bead elution</u>.   After the final washing liquid
was drawn out (Section 2.C.), 1 ml of Min A salts
solution was added to the beads.   This bead mixture was
added directly to a concentrated bacteria sample for
infection (Sections 3.A. and 3.B., following).

30

3.   <u>Amplification</u>

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A.   Preparation of plating cells. Fresh *E. coli*
(XL-1 Blue MRF') culture was grown to OD_{600} = 0.5 in LB
media containing 12.5 µg/ml of tetracycline. For each
5   panning condition, 20 ml of this culture were chilled
on ice and centrifuged. The bacterial pellet was
resuspended in 1 ml of the Min A Salts solution.

B.   Transduction. Each mixture from different
10   elution methods (Sections 2.D. and 2.E., above) was
added to a concentrated bacteria sample (Section 3.A.)
and incubated at 37°C for fifteen minutes. Two
milliliters of NZCYM media (2XNZCYM, 50 µg/ml
Ampicillin) were added to each mixture and incubated at
15   37°C for fifteen minutes. The resulting 4-ml solution
was plated on a large NZCYM agar plate containing 50
µg/ml of Ampicillin and incubated overnight at 37°C.

C.   Phage Harvesting. Each of the bacteria/phage
20   mixtures that had been grown overnight on a large NZCYM
agar plate (Section 3.B.) was scraped off in 35 ml of
LB media, and the agar plate was further rinsed with an
additional 35 ml of LB media. The resulting
bacteria/phage mixture in LB media was centrifuged to
25   remove the bacteria. Fifty milliliters of the phage
supernatant was transferred to a fresh tube, and 12.5
ml of PEG solution (20% PEG 8000, 3.5 M ammonium
acetate) were added and incubated on ice for two hours
to precipitate phages. Precipitated phage were
30   centrifuged down and resuspended in 6 ml of the phage
resuspension buffer (250 mM NaCl, 100 mM Tris pH8, 1 mM

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EDTA). This phage solution was further purified by
centrifuging away the remaining bacteria and
precipitating the phage for the second time by adding
1.5 ml of the PEG solution. After a centrifugation
5   step, the phage pellet was resuspended in 400 ml of
PBS. This solution was subjected to a final
centrifugation to rid it of any remaining bacterial
debris. The resulting phage preparation was titered by
a standard plaque formation assay (Molecular Cloning,
10   Maniatis, et al., Third Edition).

4.   Additional rounds of selection and amplification.

In a second round, the amplified phage (10^{10} pfu)
15   from the first round (Section 3.C.) was used as the
input phage to perform the selection and amplification
steps (Sections 2 and 3). The amplified phage (10^{10}
pfu) from the second round, in turn, was used as the
input phage to perform the third round of selection and
20   amplification (Sections 2 and 3). After the elution
steps (Sections 2.D., 2.E., and 2.F.) of the third
round, a small fraction of the eluted phage was plated
out as in the plaque formation assay (Section 3.C.).
Individual plaques were picked and placed into 96-well
25   microtiter plates containing 100 µl of TE buffer (10 mM
Tris, 1 mM EDTA, ph 8.0) in each well. Sambrook et
al., Molecular Cloning, A Laboratory Manual, Cold
Spring Harbor Laboratory Press, Cold Spring Harbor, NY
(1989). These master plates were incubated at 4°C
30   overnight to allow phages to elute into the TE buffer.

5.   <u>Clonal analysis (Phage ELISA and Sequencing)</u>

The phage clones were analyzed by phage ELISA and
by conventional sequencing methods. The sequences were
5   ranked based on the combined results from these two
assays.

A.   <u>Phage ELISA</u>. An XL-1 Blue MRF' culture was
grown until OD$_{600}$ reached 0.5. Thirty microliters of
10 this culture were aliquoted into each well of a 96-well
Maxisorp microtiter plate. Ten microliters of eluted
phage (from Section 4) were added to each well and
allowed to infect bacteria for fifteen minutes at room
temperature. One hundred and thirty microliters of LB
15 media containing 12.5 µg/ml of tetracycline and 50
µg/ml of ampicillin were added to each well. The
microtiter plate was then incubated with shaking
overnight at 37°C. Recombinant NGF protein (1 mg/ml in
PBS) was allowed to coat the 96-well plates (NUNC)
20 overnight at 4°C. As a control, pure streptavidin was
coated on a separate Maxisorp plate at 2 µg/ml in PBS.
On the following day, liquid in the protein-coated
Maxisorp plates was discarded, and each well was
blocked with 300 ml of 5% milk solution at 4°C
25 overnight (alternatively, one hour at room
temperature). The milk solution was discarded and the
wells were washed three times with the PBST solution.
After the last washing step, 50 µl of PBST-4% milk were
added to each well of the protein-coated Maxisorp
30 plates. Each of the 50-µl overnight cultures in the

96-well microtiter plate was transferred to the
corresponding wells of the NGF-coated plates as well as
to the control streptavidin-coated plates. The 100-μl
mixtures in the two kinds of plates were incubated for

5   one hour at room temperature. The liquid was discarded
from the Maxisorp plates and the wells were washed five
times with PBST. The HRP-conjugated anti-M13 antibody
(Pharmacia) was diluted to 1:7500, and 100 μl of the
diluted solution were added to each well of the

10 Maxisorp plates for one hour incubation at room
temperature. The liquid was again discarded and the
wells were washed seven times with PBST. One hundred
microliters of TMB substrate (Sigma) were added to each
well for the color reaction to develop, and the

15 reaction was stopped with 50 μl of 5N H_2SO_4 solution.
The OD_{450} was read on a plate reader (Molecular
Devices).

        B.   Sequencing of the phage clones. For each
20 phage clone, the sequencing template was prepared by a
PCR method. The following oligonucleotide pair was
used to amplify an approximately 500-base pair
fragment: first primer, 5'-CGGCGCAACTATCGGTATCAAGCTG-3'
(SEQ ID NO: 127), and second primer, 5'-
25 CATGTACCGTAACACTGAGTTTCGTC-3' (SEQ ID NO: 128). The
following mixture was prepared for each clone.

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Reagents	Volume (μL) per tube
dH$_2$O	26.25
50% glycerol	10
10X PCR Buffer (w/o MgCl$_2$)	5
25 mM MgCl$_2$	4
10 mM dNTP mix	1
100 μM primer 1	0.25
100 μM primer 2	0.25
Taq polymerase	0.25
Phage in TE (section 4)	3
Final reaction volume	50

A thermocycler (GeneAmp PCR System 9700, Applied Biosystems, Inc., Foster City, CA) was used to run the
5   following program: 94°C for 5 minutes; [94°C for 30 seconds, 55°C for 30 seconds, 72°C for 45 seconds] x 30 cycles; 72°C for 7 minutes; cool to 4°C. The PCR product from each reaction was purified using a QIAquick Multiwell PCR Purification kit (Qiagen),
10   following the manufacturer's protocol. The purified product was checked by running 10 μl of each PCR reaction mixed with 1 μl of 10X agarose gel loading dye on a 1% agarose gel. The remaining product was then sequenced using an ABI 377 Sequencer (Applied
15   Biosystems, Foster City, CA) in accordance with the manufacturer's recommended protocol.

6.   Peptide sequence ranking and consensus sequences

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A.   Sequence ranking. The phage clones were
ranked by the combined results of the phage ELISA and
DNA sequencing. Peptide sequences that occurred

5   multiple times were considered candidates for
modification. In addition, each of the peptide
encoding nucleotide sequences (Section 5.B.) were
correlated to ELISA data. The peptides expressed by
phage clones yielding higher OD_{450} readings in the NGF-

10   coated wells relative to the OD_{450} readings they
produced in the corresponding streptavidin-coated wells
were also considered for modification. A monomer of
each peptide sequence selected for modification based
on these criteria (SEQ ID NOS:1-29) was fused in-frame

15   to the Fc region of human IgG1 (i.e., modified
peptides) as described in Example 2.

B.   Consensus sequence determination. From the
TN8-IX library, two different consensus sequences were

20   determined. They were: X C W F/W S/T E E G C X X X
(SEQ ID NO:274), and X L/F Q C X F/Y S X X G C P X X
(SEQ ID NO:275). The underlined "core amino acid
sequences" were obtained by determining the most
frequently occurring amino acid in each position. The

25   two cysteines adjacent to the core sequences were fixed
amino acids in the TN8-IX library. An LQS motif
followed by the fixed cysteine was observed in many
sequences from the TN12-I library:
X X X C X X X X X X X L Q S C X X X (SEQ ID NO:276).

30   However, there was no highly conserved motif found in
the sequences obtained from the Linear library.

Example 2:   Construction of Peptide-Fc Fusion Products

    The candidate peptide sequences selected as
5   described above were used to construct fusion proteins
    in which a monomer of each peptide was fused in-frame
    to the Fc region of human IgG1.   Each modified peptide
    was constructed by annealing the pairs of
    oligonucleotides ("oligos") indicated in Table 3 to
10 generate a duplex encoding the peptide and a linker
    comprised, depending on the peptide, of five glycine
    residues, one leucine residue and one glutamic acid
    residue as an NdeI to XhoI fragment.   These duplex
    molecules were ligated into a vector (pAMG21-Fc N-
15 terminal, described further below) containing the human
    Fc gene, also digested with NdeI and XhoI.   The
    resulting ligation mixtures were transformed by
    electroporation into E. coli strain 2596 cells (GM221,
    described further below).   Clones were screened for the
20 ability to produce the recombinant protein product and
    to possess the gene fusion having a correct nucleotide
    sequence.   A single such clone was selected for each of
    the modified peptides (i.e., Fc-peptide fusion
    products).   The peptide portions of samples NGF-C12
25 through NGF-C18 were consensus sequences based on the
    analysis described above.

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Table 3

Anti-NGF Peptides and Oligonucleotides used to Generate
Modified Anti-NGF Peptides

SEQ ID NO:	Sequence of Peptide Portion of Fc-Peptide Fusion Product	SEQ ID NO: for Sense oligo	SEQ ID NO: for Anti-sense oligo
1	TGYTEYTEEWPMGFGYQWSF	149	150
2	TDWLSDFPFYEQYFGLMPPG	151	152
3	FMRFPNPWKLVEPPQGWYYG	153	154
4	VVKAPHFEFLAPPHFHEFPF	155	156
5	FSYIWIDETPSNIDRYMLWL	157	158
6	VNFPKVPEDVEPWPWSLKLY	159	160
7	TWHPKTYEEFALPFFVPEAP	161	162
8	WHFGTPYIQQQPGVYWLQAP	163	164
9	VWNYGPFFMNFPDSTYFLHE	165	166
10	WRIHSKPLDYSHVWFFPADF	167	168
11	FWDGNQPPDILVDWPWNPPV	169	170
12	FYSLEWLKDHSEFFQTVTEW	171	172
13	QFMELLKFFNSPGDSSHHFL	173	174
14	TNVDWISNNWEHMKSFFTED	175	176
15	PNEKPYQMQSWFPPDWPVPY	177	178
16	WSHTEWVPQVWWKPPNHFYV	179	180
17	WGEWINDAQVHMHEGFISES	181	182
18	VPWEHDHDLWEIISQDWHIA	183	184
19	VLHLQDPRGWSNFPPGVLEL	185	186
20	IHGCWFTEEGCVWQ	129	130
21	YMQCQFARDGCPQW	131	132
22	KLQCQYSESGCPTI	133	134
23	FLQCEISGGACPAP	135	136
24	KLQCEFSTSGCPDL	137	138
25	KLQCEFSTQGCPDL	139	140
26	KLQCEFSTSGCPWL	141	142
27	IQGCWFTEEGCPWQ	143	144
28	SFDCDNPWGHVLQSCFGF	145	146
29	SFDCDNPWGHKLQSCFGF	147	148

5

Construction of pAMG21-Fc N-Terminal Vector

pAMG21

Expression plasmid pAMG21 (ATCC No. 98113) is

10 derived from expression vector pCFM1656 (ATCC No.

69576) and the expression vector system described in
United States Patent No. 4,710,473, by following the
procedure described in published International Patent
Application WO 00/24782 (see the portion of Example 2
5   therein extending from pages 100-103, as well as
Figures 17A and 17B).

Fc N-terminal Vector

10   The DNA sequence of the pAMG21-Fc N-terminal
vector inserted into expression plasmid pAMG21 between
the NdeI and BamHI restriction sites is shown in
Figure 3; top strand, SEQ ID NO: 61, bottom strand SEQ
ID NO: 62.

15   The DNA sequences encoding the thirty peptides
(SEQ ID NOS: 1-29) generated for splicing into the
above vector and expression as Fc-peptide fusion
products are represented by SEQ ID NOS: 63 to 91,
inclusive. These DNA sequences include a codon for
20   Met^{-1} (this feature is optional).

The sequences of the corresponding "methionyl
mature" peptides encoded by these DNA sequences, i.e.,
expressed with a methionine residue at the N-terminus,
are represented by SEQ ID NOS: 92-120, inclusive.

25   In addition to making these modified peptides as
N-terminal fusions to Fc, some of them were also made as
C-terminal fusion products. The vector used for making
the C-terminal fusion products is described below.

30   ## Fc C-terminal vector

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The DNA sequence of the pAMG21-Fc C-terminal
vector inserted into expression plasmid pAMG21 between
the NdeI and BamHI restriction sites is shown in Figure
4; top strand, SEQ ID NO: 121, bottom strand, SEQ ID
5   NO: 122.

GM221 (#2596).   Host strain #2596, used for expressing
Fc-peptide fusion proteins, is an *E. coli* K-12 strain
modified to contain both the temperature sensitive
10   lambda repressor cI857s7 in the early *ebg* region and
the lacI^Q repressor in the late *ebg* region.   The
presence of these two repressor genes allows the use of
this host with a variety of expression systems, but the
repressors are irrelevant to expression from luxP$_R$.
15   Details regarding its construction are found in WO
00/24782 (see Example 2 therein).

Expression in *E. coli*.   Cultures of each of the pAMG21-
Fc fusion constructs in *E. coli* GM221 were grown at
20   37ºC in Terrific Broth medium (See Tartof and Hobbs,
"Improved media for growing plasmid and cosmid clones",
Bethesda Research Labs Focus, Volume 9, page 12, 1987,
cited in aforementioned Sambrook et al. reference).
Induction of gene product expression from the luxPR
25   promoter was achieved following the addition of the
synthetic autoinducer, N-(3-oxohexanoyl)-DL-homoserine
lactone, to the culture medium to a final concentration
of 20 nanograms per milliliter (ng/ml).   Cultures were
incubated at 37ºC for an additional six hours.   The
30   bacterial cultures were then examined by microscopy for
the presence of inclusion bodies and collected by

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centrifugation. Refractile inclusion bodies were
observed in induced cultures, indicating that the
Fc-fusions were most likely produced in the insoluble
fraction in *E. coli*. Cell pellets were lysed directly
5   by resuspension in Laemmli sample buffer containing 10%
β-mercaptoethanol and then analyzed by SDS-PAGE. In
each case, an intense coomassie-stained band of the
appropriate molecular weight was observed on an
SDS-PAGE gel.

10

Purification. Cells were broken in water (1/10) using
high pressure homogenization (two passes at 14,000
PSI), and inclusion bodies were harvested by
centrifugation (4000 RPM in a J-6B centrifuge, for one
15   hour). Inclusion bodies were solubilized in 6 M
guanidine, 50 mM Tris, 10 mM DTT, pH 8.5, for one hour
at a 1/10 ratio. For linear peptides fused to Fc, the
solubilized mixture was diluted twenty-five times into
2 M urea, 50 mM Tris, 160 mM arginine, 2 mM cysteine,
20   pH 8.5. The oxidation was allowed to proceed for two
days at 4°C, allowing formation of the disulfide-linked
compound (i.e., Fc-peptide homodimer). For cyclic
peptides fused to Fc, this same protocol was followed
with the addition of the following three folding
25   conditions: (1) 2 M urea, 50 mM Tris, 160 mM arginine,
4 mM cysteine, 1mM cystamine, pH 8.5; (2) 4 M urea,
20% glycerol, 50 mM Tris, 160 mM arginine, 2 mM
cysteine, pH 8.5; and (3) 4 M urea, 20% glycerol, 50 mM
Tris, 160 mM arginine, 4 mM cysteine, 1mM cystamine, pH
30   8.5. The refolded protein was dialyzed against 1.5 M
urea, 50mM NaCl, 50mM Tris, pH 9.0. The pH of this

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mixture was lowered to pH 5 with acetic acid. The
precipitate was removed by centrifugation, and the
supernatant was adjusted to a pH of from 5 to 6.5,
depending on the isoelectric point of each fusion

5   product. The protein was filtered and loaded at 4°C
onto an SP-Sepharose HP column equilibrated in 20 mM
NaAc, 50 mM NaCl at the pH determined for each
construct. The protein was eluted using a 20-column
volume linear gradient in the same buffer ranging from

10   50 mM NaCl to 500 mM NaCl. The peak was pooled and
filtered.

**Example 3: *In vitro* NGF-Inhibition Activity of
Modified Peptides**

15

The effectiveness of the modified peptides (Fc
fusion products) prepared in Example 2 as inhibitors of
NGF activity (i.e., NGF "neutralization") was evaluated
by measuring the ability of each modified peptide to

20   block NGF induction of vanilloid receptor-1 (VR1)
expression.

Dorsal Root Ganglion Neuronal Cultures. Dorsal root
ganglia (DRG) were dissected one by one under aseptic

25   conditions from all spinal segments of embryonic 19-day
old (E19) rats that were surgically removed from the
uterus of timed-pregnant, terminally anesthetized
Sprague-Dawley rats (Charles River, Wilmington, MA).
DRG were collected in ice-cold L-15 media (GibcoBRL,

30   Grand Island, NY) containing 5% heat inactivated horse
serum (GibcoBRL), and any loose connective tissue and

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blood vessels were removed. The DRG were rinsed twice
in Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate buffered
saline (DPBS), pH 7.4 (GibcoBRL). The DRG were then
dissociated into single cell suspension using a papain

5   dissociation system (Worthington Biochemical Corp.,
Freehold, NJ). Briefly, DRG were incubated in a
digestion solution containing 20 U/ml of papain in
Earle's Balanced Salt Solution (EBSS) at 37°C for fifty
minutes. Cells were dissociated by trituration through

10   fire-polished Pasteur pipettes in a dissociation medium
consisting of MEM/Ham's F12, 1:1, 1 mg/ml ovomucoid
inhibitor and 1 mg/ml ovalbumin, and 0.005%
deoxyribonuclease I (DNase). The dissociated cells
were pelleted at 200 x g for five minutes and re-

15   suspended in EBSS containing 1 mg/ml ovomucoid
inhibitor, 1 mg/ml ovalbumin and 0.005% DNase. Cell
suspension was centrifuged through a gradient solution
containing 10 mg/ml ovomucoid inhibitor, 10 mg/ml
ovalbumin at 200 x g for six minutes to remove cell

20   debris, and then filtered through a 88-μm nylon mesh
(Fisher Scientific, Pittsburgh, PA) to remove any
clumps. Cell number was determined with a
hemocytometer, and cells were seeded into poly-
ornithine 100 μg/ml (Sigma, St. Louis, MO) and mouse

25   laminin 1 μg/ml (GibcoBRL)-coated 96-well plates at 10
x 10^3 cells/well in complete medium. The complete
medium consisted of minimal essential medium (MEM) and
Ham's F12, 1:1, penicillin (100 U/ml), streptomycin
(100 μg/ml), and 10% heat inactivated horse serum

30   (GibcoBRL). The cultures were kept at 37°C, 5% CO_2 and
100% humidity. For controlling the growth of non-

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neuronal cells, 5-fluoro-2'-deoxyuridine (75 μM) and

uridine (180 μM) were included in the medium.

Treatment with NGF and anti-NGF. Two hours after

5   plating, cells were treated with recombinant human β-

NGF or recombinant rat β-NGF at a concentration of 10

ng/ml (0.38 nM). Positive controls comprising serial-

diluted anti-NGF antibody (R&D Systems, Minneapolis,

MN) were applied to each culture plate. Modified

10   peptides (from Example 2) were added at ten

concentrations using 3.16-fold serial dilutions. All

of the samples were diluted in complete medium before

being added to the cultures. Incubation time was 40

hours prior to measurement of VR1 expression.

15

Measurement of VR1 Expression in DRG Neurons. Cultures

were fixed with 4% paraformaldehyde in Hanks' balanced

salt solution for fifteen minutes, blocked with

Superblock (Pierce, Rockford, IL), and permeabilized

20   with 0.25% Nonidet P-40 (Sigma) in Tris.HCl (Sigma)-

buffered saline (TBS) for one hour at room temperature.

Cultures were rinsed once with TBS containing 0.1%

Tween 20 (Sigma) and incubated with rabbit anti-VR1 IgG

(prepared at Amgen) for one and one-half hours at room

25   temperature, followed by incubation of Eu-labeled anti-

rabbit second antibody (Wallac Oy, Turku, Finland) for

one hour at room temperature. Washes with TBS (3 x

five minutes with slow shaking) were applied after each

antibody incubation. Enhance solution (150 μl/well,

30   Wallac Oy) was added to the cultures. The fluorescence

signal was then measured in a time-resolved fluorometer

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(Wallac Oy). VR1 expression in samples treated with the modified peptides was determined by comparing to a standard curve of NGF titration from 0-1000 ng/ml. Percent inhibition (compared to maximum possible

5　inhibition) of NGF effect on VR1 expression in DRG neurons was determined by comparing to controls that were not NGF-treated. Results are given in Table 4. The effectiveness of a sampling of the peptides identified in Example 1, Section 6, paragraph A was

10　also determined in the manner described above (data not shown).

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Table 4

Neutralizing Activity of Modified Peptides *In Vitro*

SEQ ID NO:	Peptide Portion of Fusion Product	Attachment of Peptide Portion to Fc	Activity of Modified Peptide
1	TGYTEYTEEWPMGFGYQWSF		−
2	TDWLSDFPFYEQYFGLMPPG		+
2	TDWLSDFPFYEQYFGLMPPG	C-term	−
3	FMRFPNPWKLVEPPQGWYYG		n/a
4	VVKAPHFEFLAPPHFHEFPF		+
4	VVKAPHFEFLAPPHFHEFPF	C-term	−
5	FSYIWIDETPSNIDRYMLWL		−
6	VNFPKVPEDVEPWPWSLKLY		+
6	VNFPKVPEDVEPWPWSLKLY	C-term	+
7	TWHPKTYEEFALPFFVPEAP		+
8	WHFGTPYIQQQPGVYWLQAP		+
9	VWNYGPFFMNFPDSTYFLHE		+
10	WRIHSKPLDYSHVWFFPADF		+
10	WRIHSKPLDYSHVWFFPADF	C-term	+
11	FWDGNQPPDILVDWPWNPPV		+
11	FWDGNQPPDILVDWPWNPPV	C-term	−
12	FYSLEWLKDHSEFFQTVTEW		n/a
13	QFMELLKFFNSPGDSSHHFL		−
14	TNVDWISNNWEHMKSFFTED		+
14	TNVDWISNNWEHMKSFFTED	C-term	+
15	PNEKPYQMQSWFPPDWPVPY		−
16	WSHTEWVPQVWWKPPNHFYV		n/a
17	WGEWINDAQVHMHEGFISES		−
18	VPWEHDHDLWEIISQDWHIA		+
18	VPWEHDHDLWEIISQDWHIA	C-term	−
19	VLHLQDPRGWSNFPPGVLEL		n/a
20	IHGCWFTEEGCVWQ		n/a
21	YMQCQFARDGCPQW		+
22	KLQCQYSESGCPTI		+
23	FLQCEISGGACPAP		−
24	KLQCEFSTSGCPDL		+
25	KLQCEFSTQGCPDL		+
26	KLQCEFSTSGCPWL		+
27	IQGCWFTEEGCPWQ		+
28	SFDCDNPWGHVLQSCFGF		−
29	SFDCDNPWGHKLQSCFGF		−

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"+" indicates at least 50% inhibition of NGF
induced activity observed at modified peptide
concentrations of 18 μM or lower.

"-" indicates less than 50% inhibition of NGF
5   induced activity observed at modified peptide
concentrations of at least 18 μM.

"n/a" means not applicable.

Peptides are attached to the N-terminus of Fc unless
designated "C-term" wherein such peptides are attached
10   to the C-terminus of Fc.

**Example 4: Identification of Additional Peptides
Capable of Modulating NGF Activity**

15   I.   Construction of Secondary anti-NGF Peptide
Libraries

A.   Electrocompetent E. coli cells. *E. coli* XL1-
Blue MRF' electroporation competent cells were
20   purchased from Stratagene Cloning Systems, La Jolla,
California (catalog no. 200158).

B.   Modification of pCES1 vector. A PCR reaction
was performed using Extend Long Template PCR Systems
25   (Roche Diagnostics Corp., Indianapolis, IN) with 1 μg of
pCES1 vector (TargetQuest Inc., now Dyax Corp.,
Cambridge, MA) as a template. The volume of PCR mixture
was 100 μl and contained 1x PCR buffer, 200 nM of each
of the primers, 5'-CAAACGAATGGATCCTCATTAAAGCCAGA-3' (SEQ
30   ID NO: 191) and 5'-
GGTGGTGCGGCCGCACTCGAGACTGTTGAAAGTTGTTTAGCA-3' (SEQ ID
NO: 192), 200 nM dNTP, and 3 units of Tag DNA
polymerase. A TRIO-Thermoblock (Biometra, Göttingen,
Germany) PCR system was used to run the following

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program: 94°C for 5 minutes; 30 cycles of [94°C for 30

seconds, 50°C for 30 seconds, 72°C for 45 seconds]; 72°C

for 10 minutes; cool to 4°C. The PCR products were run

on a 1% agarose gel and purified with a Qiagen Spin

5   Column (Qiagen Inc., Valencia, CA) in accordance with

the manufacturer's protocols. A second PCR reaction was

performed with 5 µl of PCR products and 200 nM of each

of the two primers, 5'-CAAACGAATGGATCCTCATTAAAGCCAGA-3'

and 5'-AACACAAAAGTGCACAGGGTGGAGGTGGTGGTGCGGCCGCACT-3'

10   (SEQ ID NOS: 191 and 193, respectively), using the same

PCR conditions as described above. The PCR products and

original pCES1 vector were digested separately in a 100-

µl reaction containing 1x NEB2 buffer, 60 units of ApaLI

(New England Biolabs, Beverly, MA), 60 units of BamHI

15   (New England Biolabs) at 37°C for one hour. Both

digested DNA molecules were purified with a Qiagen Spin

Column and ligated together in a 40-ul reaction

containing 1x ligation buffer and 40 units of T4 DNA

ligase (New England Biolabs) at room temperature,

20   overnight.

      The vectors were transfected into *E. coli* cells and

incubated at 37°C, overnight. Isolated single colonies

were selected, and plasmid was purified with Qiagen Spin

Column. The correct insert was confirmed by DNA

25   sequencing.

      C.   Preparation of vector DNA. One microgram of

the modified pCES1 vector DNA (Section I.B., above) was

transformed into 40 µl of electrocompetent XL1-blue *E.*

30   *coli* (Section I.A.) using a Gene Pulser II (Bio-Rad

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Labs, Hercules, CA) with the setting of 2500 V, 25 μF,
and 200 ohms. The transformed bacteria sample was then
transferred immediately into a tube containing 960 μl
of SOC (2% tryptone, 0.5% yeast extract, 10 mM NaCl,

5   2.5 mM KCl, 20 mM glucose, 10 mM MgSO₄, 10mM MgCl₂),
and this culture was allowed to grow at 37°C, with
shaking, for one hour. The cells were then spread onto
a plate of 2xYT agar supplemented with 100 μg/ml of
ampicillin, 12.5 μg/ml of tetracycline and 2% glucose

10   (2xYTAGT; Invitrogen Corporation, Carlsbad, CA) and
incubated at 37°C overnight. A single colony was
confirmed by sequencing and used to inoculate two
liters of 2xYTAGT media at 37°C, with shaking,
overnight. The plasmid vector DNA was purified with a

15   Qiagen Plasmid Maxi Kit according to the manufacturer's
protocols.

         D. Digestion of vector DNA. A total of about
1,100 micrograms of vector DNA was digested in two

20   batches. Eight hundred micrograms of vector DNA
(Section I.C.) was digested at 37°C in a 1500 μl
reaction containing 1x NEB buffer 2, 1000 units of
ApaLI and 1000 units of XhoI, overnight. The remaining
300 micrograms of vector DNA (Section I.C.) was

25   digested in a 500-μl reaction containing 1x NEB buffer
2, 300 units of ApaLI, and 300 units of XhoI at 37°C
overnight. Both restriction digest reactions were
incubated overnight at 37°C and analyzed in a pre-made
0.8% agarose gel (Embi Tec, San Diego, CA). The

30   linearized vector DNA was excised from the gel and

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extracted with QIAquick Gel Extraction Kit (Qiagen
Inc.) in accordance with the manufacturer's directions.

      E.   Preparation of library oligonucleotides. Six
5   library oligonucleotides (two fixed and four doped)
were designed based on the sequences derived from
previous work. The two fixed library oligonucleotides
were 5'-CACAGTGCACAGGGTNNKNNKNNKNNKNNKNNKNNKCTGCAGNNKS
ARTWTAGCNNKNNKNNKNNKNNKNNKNNKCATTCTCTCGAGATCA-3' and
10 5'-CACAGTGCACAGGGTNNKNNKNNKAAACTGCAGNNKGAATTTAGCACCAGC
GGCNNKCCGGATCTGNNKNNKNNKCATTCTCTCGAGATCA-3' (SEQ ID
NOS:194 and 195, respectively; N and K represents an
equal representation of nucleotides A,G,C,T and G,T
during oligo synthesis, respectively,). The three 70%-
15 doped library oligo-nucleotides were 5'-CACAGTGCACAGGGT
NNKNNKNNKNNKNNKNNKNNKtgKttKacKgaKgaKggKNNKNNKNNKNNKNNKN
NKNNKCATTCTCTCGAGATCA-3', 5'-CACAGTGCACAGGGTNNKttKtgKga
KggKaaKcaKccKccKgaKatKttKgtKgaKtgKccKtgKaaKccKccKgtKNNK
CATTCTCTCGAGATCA-3', and 5'-CACAGTGCACAGGGTNNKacKgaKtg
20 KctKagKgaKttKccKttKtaKgaKcaKtaKttKggKctKatKccKccKggKNN
KCATTCTCTCGAGATCA-3' (SEQ ID NOS: 196, 197, and 198,
respectively; lower case letters represent a mixture of
70% of the indicated base and 10% of each of the other
three nucleotides) The 91% doped library oligo was
25 5'-CACAGTGCACAGGGTNNKNNKNNKaaKctKcaKNNKgaKttKtcKacKtcKg
gKNNKccKgaKctKNNKNNKNNKCATTCTCTCGAGATCA-3' (SEQ ID NO:
199; lower case letters represent a mixture of 91% of
the indicated base and 3% of each of the other three
nucleotides). All were synthesized by the Amgen DNA
30 synthesis group. Each of these oligonucleotides was

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used as a template in a Polymerase Chain Reaction
(PCR).

An Expand High Fidelity PCR System kit (Roche
Diagnostics Corp.) was used for PCR reactions. Each
5   PCR reaction comprised 2400 µl in volume, and half of
the volume contained 1 nM of a library oligonucleotide
while the other half contained 10 nM of a library
oligonucleotide, 1X PCR buffer, 300 nM of each of the
primers, 5'-CACAGTGCACAGGGT-3' (SEQ ID NO: 200) and 5'-
10 TGATCTCGAGAGAATG-3' (SEQ ID NO: 201), 200 µM dNTP, 2 mM
MgCl$_2$, and 84 units of Expand polymerase. A
thermocycler (GeneAmp PCR System 9700, Applied
Biosystems, Foster City, CA) was used to run the
following program: 94°C for 5 minutes; 30 cycles of
15 [94°C for 30 seconds, 55°C for 30 seconds, 72°C for 45
seconds]; 72°C for 7 minutes; cool to 4°C. The free
nucleotides were removed using the QIAquick PCR
Purification Kit (Qiagen Inc., Catalog No. 28104) in
accordance with the manufacturer's protocols.
20

F. Digestion of library oligonucleotides. Each
of the PCR products from the 2400-µl reaction (Section
I.E.) was digested in a 500 µl reaction that contained
1x NEB buffer2, 250 units of ApaLI, and 250 units of
25 XhoI at 37°C, overnight. The digested DNA was
separated on a pre-made 3% agarose gel (Embi Tec). The
DNA band of interest from each reaction was cut from
the gel and extracted with a QIAquick Gel Extraction
Kit.

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G.   Ligation of vector with library
oligonucleotides. The linearized vector (Section I.D.)
and each digested PCR product (Section I.F.) were
ligated at a 1:5 molar ratio in two batches: the first
5   batch included one fixed library oligonucleotide and
three doped library oligonucleotides in a 800 µl
reaction containing 1x NEB ligation buffer and 20,000
units of the T4 DNA ligase at 16°C, overnight; the
second batch included two fixed library oligo-
10 nucleotides and one doped library oligonucleotide in a
400 µl reaction containing 1x NEB ligation buffer and
20,000 units of T4 DNA ligase at 16°C, overnight. The
ligated products were incubated at 65°C for twenty
minutes to inactivate the T4 DNA ligase and further
15 incubated with 100 units of NotI at 37°C for two hours
to minimize vector self-ligation. The ligated products
were then purified by a standard phenol/chloroform
extraction (Molecular Cloning, Maniatis, et al., 3rd
Edition) and resuspended in 100 µl of water.
20

H.   Electroporation Transformation. For each
library, ten electroporation reactions were performed.
For each transformation, 10 µl of ligated vector DNA
(Section I.G.) and 300 µl of XL1-BLUE MRF' cells
25 (Section I.A.) were mixed in a 0.2-cm cuvette (Bio-Rad
Labs). The resulting mixture was pulsed with a Gene
Pulser II, with a setting of 2500 V, 25 µF, and 200
ohms. The transformed bacteria from the ten
electroporation reactions were combined and transferred
30 into a flask containing 30 ml of SOC for incubation at

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37°C for one hour. The cells were then added to 400 ml

of 2xYTAGT and grown at 37°C, with shaking, for five

hours. The cells were then centrifuged at 4000 rpm for

fifteen minutes at 4°C. The cell pellets were then

5   resuspended in 12 ml of 2xYT broth containing 15%

glycerol and stored at -80°C. This was the primary

stock for the libraries. Titers showed library sizes

of 2.5×10^9 (library number 93), 2.5×10^9 (library number

59), 5.0×10^9 (library number 72), 8.0×10^9 (library

10   number 95), 8.5×10^9 (library number 39) independent

transformants and 1.5×10^9 (library number 63)

independent transformants for the fixed and doped

libraries, respectively.

15   II.   Amplification of the Libraries.

     A.   Making secondary stock of the libraries. The

primary library cell stock (Section I.H.) was used to

inoculate 1700 ml (for both fixed libraries 93 and 59),

20   and 1800 ml, 2700 ml, 3000 ml, 1000 ml (for doped

libraries 72, 95, 39 and 63, respectively) of 2xYTAGT

media so that the starting OD_{600} was equal to 0.1. The

cultures were allowed to grow at 37°C, with shaking,

for several hours until the OD_{600} was 0.5. A one-tenth

25   aliquot from each library were taken out and grown up

in separate flasks for another two hours at 37°C.

These sub-cultures were centrifuged at 4000 rpm (using

a Beckman JA-14 rotor) for 10 minutes at 4°C, and the

bacteria pellets (for each library) were resuspended in

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9.5 ml of 2xYT containing 15% glycerol/for storage at
-80°C.

　　　B.　　Phage Induction.　M13KO7 helper phage
5　　aliquots (Amersham Biosciences, Inc., Piscataway, NJ)
　　were added to the remaining bacteria cultures at OD_{600} =
　　0.5 (Section II.A.) to a final concentration of 3 x 10^9
　　pfu/ml.　The helper phages were allowed to infect
　　bacteria at 37°C for thirty minutes without shaking and
10　　thirty minutes with slow shaking.　The infected cells
　　were centrifuged with 5000 rpm for fifteen minutes at
　　4°C.　The cell pellets were resuspended in the same
　　volume (Section II.A.) with 2YT broth with 100 μg/ml of
　　ampicillin, 40 μg/ml of kanamycin and 12.5 μg/ml of
15　　tetracycline (2xYTAKT).　The phagemid production was
　　allowed to occur at 30°C overnight while shaking.

　　　C.　　Harvest of phage.　The bacteria cultures
　　(Section II.B.) were centrifuged at 5000 rpm for
20　　fifteen minutes at 4°C.　The supernatants were
　　transferred into new bottles, and 0.2 volume of 20%
　　PEG/2.5M NaCl was added and incubated on ice for one
　　hour to precipitate the phagemids.　Precipitated
　　phagemids were centrifuged at 10,000 rpm for thirty
25　　minutes at 4°C and carefully resuspended with 100 ml of
　　cold PBS.　The phagemid solution was further purified
　　by centrifuging away the remaining cells with 4000 rpm
　　for ten minutes at 4°C and precipitating the phagemids
　　by adding 0.2 volume of 20% PEG/2.5M NaCl.　The
30　　phagemids were centrifuged at 10,000 rpm for thirty

minutes at 4°C, and the phagemid pellets were
resuspended using 18 ml of cold PBS. Six milliliters
of 60% glycerol solution were added to the phagemid
solution for storage at -80°C. The phagemid titers

5   were determined by a standard procedure (see Molecular
Cloning, Maniatis, et al., 3rd Edition).

III.   Selection of Human NGF Binding Phages.

10   A.   Biotinylation of human NGF. One milligram of
human NGF was biotinylated using an EZ-Link Sulfo-NHS-
LC-Biotinylation Kit (Pierce, Rockford, IL) in
accordance with the manufacturer's directions.

15   B.   Immobilization of NGF on magnetic beads.
Biotinylated NGF (Section III.A.) was immobilized on
Dynabead M-280 Streptavidin (DYNAL, Lake Success, NY)
at a concentration of 200 ng NGF per 100 µl of bead
stock from the manufacturer. After drawing the beads

20   to one side of a tube using a magnet and then pipetting
away the liquid, the beads were washed twice with the
phosphate buffer saline (PBS) and resuspended in PBS.
The biotinylated NGF protein was added to the washed
beads at the above concentration and incubated, with

25   rotation, for one hour at room temperature. The NGF-
coated beads were then blocked by adding BSA to 2%
final concentration and incubating overnight at 4°C,
with rotation. The resulting NGF-coated beads were
washed twice with PBST (PBS with 0.05% Tween-20) before

30   being subjected to the selection procedures described
in the next section.

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C.   Selection using the NGF-coated beads.

Approximately 1000-fold library equivalent phagemids
(Section II.C.) were blocked for one hour with 1 ml of
5   PBS containing 2% BSA. The blocked phagemid sample was
subjected to two negative selection steps by adding to
blank beads (the same beads as Section III.B. but
without an NGF coating), and this mixture was incubated
at room temperature for fifteen minutes, with rotation.
10   The phagemid containing supernatant was drawn out using
a magnet and transferred to a second tube containing
blank beads, and this mixture was incubated at room
temperature for fifteen minutes, with rotation. The
phagemid-containing supernatant was drawn out using
15   magnet and transferred to a new tube containing NGF-
coated beads (Section III.B.), and this mixture was
incubated at room temperature for one hour, with
rotation. After the supernatant was discarded, the
phagemid-bound beads were washed ten times with 2%milk-
20   PBS; ten times with 2% BSA-PBS; ten times with PBST,
and twice with PBS. The phagemids (from libraries 72,
95, 39) were then allowed to elute in 1 ml of 100 mM
triethylamine solution (Sigma, St. Louis, MO) for ten
minutes on a rotator. The pH of the phagemid-
25   containing solution was neutralized by adding 0.5 ml of
1 M Tris-HCl (pH 7.5). The phagemids (from libraries
93, 59, 63) were eluted in 1 ml of 100 nM, 1000 nM and
100 mM TEA sequentially. The resulting phagemids were
used to infect 5 ml of freshly grown XL1-Blue MRF'
30   bacteria (OD_{600}=about 0.5) at 37°C for thirty minutes
without shaking and thirty minutes with slow shaking.

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All of the infected XL1-BLUE MRF' cells were plated on
a large 2xYTAG plate and incubated at 30°C overnight.

        D.   Induction and harvesting of phage. A 10 ml
5   aliquot of 2xYTAGT media was added to the plate
     (Section III.C.) to resuspend XL1-BLUE MRF' cells. All
     XL1-BLUE MRF' cells were collected in a tube, and a 250
     μl aliquot of these cells was added to 25 ml of 2xYTAGT
     and grown at 37°C until the OD_{600} was equal to 0.5.
10   M13KO7 helper phages were added to a concentration of
     3×10^9 cfu/ml and incubated at 37°C for thirty minutes
     without shaking and 30 minutes with slow shaking. The
     cells were centrifuged with 5000 rpm for 10 minutes at
     4°C and resuspended with 25 ml of 2xYTAK. The bacteria
15   were allowed to grow at 30°C overnight, with shaking.
     The induced phagemids were harvested and purified as
     described in Section II.C.

        E.   Second round selection. The second round
20   selection was performed as outlined in Sections III.B.
     and III.C., except as follows. Approximately 100-fold
     library equivalent phagemids resulting from Section
     III.D. were used as the input phagemid.

25   F.   Third round selection. A third round
     selection was performed as outlined in Sections III.B.
     and III.C., except as follows. Approximately 10-fold
     library equivalent phagemids resulting from Section
     III.E. was used as the input phagemid. Only 20 ng of
30   biotinylated NGF (Section III.A.) was used to coat the
     Dynabead M-280 Streptavidin. The phage-bound beads

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were washed ten times with 2% milk-PBS; ten times with

2% BSA-PBS; and ten times with PBST, in which the final

wash involved thirty minutes of incubation at room

temperature in PBST.   The beads were washed twice with

5   PBS.

     G.   Fourth round selection.   A fourth round

selection was performed as outlined in Sections III.B.

and III.C., except for the following.   Approximately

10   one-fold library equivalent phagemids resulting from

Section 3.F. were used as the input phagemid.   Only two

nanograms of biotinylated NGF (Section III.A.) were

used to coat the Dynabead M-280 Streptavidin.   The

phage-bound beads were same as in round three.

15

IV. Clonal Analysis

     A.   Preparation of master plate.   Single colonies

from the second round selection were picked and

20   inoculated into 96-well plates containing 120 µl of

2xYTAGT per well.   The 96-well plates were incubated at

30°C in a shaker overnight. Forty microliters of 60%

glycerol were added per well for storage at -80°C.

25   B.   Phagemid deep-well ELISA.   About 20 µl

aliquots of cells from the master plate (Section IV.A.)

were inoculated into a fresh Costar® 96-well two

milliliter assay block (Corning Inc., Corning, NY,

Catalog No. 3960) containing 500 µl of 2xYTAGT per

30   well, and this new plate of cells was grown at 37°C

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until the OD_{600} was approximately equal to 0.5. Forty
microliters of 2xYTAGT containing M13KO7 helper phage
(1.5×10^{10} cfu/ml) were added to each well, and the
96-well plate was incubated at 37°C for thirty minutes

5   without shaking and another thirty minutes with slow
shaking. The plate was centrifuged at 2000 rpm
(Beckman CS-6R tabletop centrifuge) for ten minutes at
4°C. The supernatants were removed from the wells, and
each cell pellet was resuspended using 500 µl of

10   2xYTAKT per well. The plate was incubated at 30°C
overnight for phagemid expression.

Human NGF was coated onto the 96-well Maxisorp
plate (NUNC) at a concentration of 5 µg/ml in 1x PBS at
4°C, overnight. As a control, BSA (Sigma) was coated

15   onto a separate Maxisorp plate at 5 µg/ml.

On the following day, the overnight cell cultures
were centrifuged at 2000 rpm for ten minutes at 4°C.
Twenty microliters of supernatant from each well were
transferred to a new 96-well plate containing a BSA/PBS

20   solution so as to dilute the supernatant at 1:2 to 1:10
range. The resulting mixtures were incubated for one
hour at room temperature, with shaking, to block the
phagemids. Meanwhile, the NGF-coated plate was blocked
with 200 µl of 2% BSA/PBS solution per well for one

25   hour at room temperature, while shaking. The BSA
solution was discarded, and each well was washed three
times with PBS solution. After the final washing step,
100 µl of blocked phagemid solution were added to each
well of the NGF-coated plate as well as the control

30   plate and incubated for one hour at room temperature

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with shaking. The liquid was discarded, and each well
was washed three times with PBST solution. One hundred
microliters of the HRP-conjugated anti-M13 mAb
(Amersham Biosciences, Inc., Piscataway, NJ) at 5,000

5   dilution were added to each well of the NGF-coated and
control plates, and these plates were incubated for one
hour at room temperature, with shaking. The liquids
were discarded again, and each well was washed three
times with PBST solution. One hundred microliters of

10   LumiGLO chemiluminescent substrates (Kirkegaard & Perry
Laboratories, Gaithersburg, MD) were added to the
wells, and each well was read by Luminoskan Ascent
DLRearly machine (Labsystems, Franklin, MA).

15   C.   Sequencing of the phage clones. PCR reaction
was performed using 1 µl of bacteria from each well of
the master plate (Section IV.A.) as a template. The
volume of each PCR mixture was 50 µl, containing 1x PCR
buffer, 300 nM of each of the primers, 5'-

20   GTTAGCTCACTCATTAGGCAC-3' (SEQ ID NO:281)and 5'-
GTACCGTAACACTGAGTTTCG-3' (SEQ ID NO: 282) , 200 µM
dNTP, 2 mM $MgCl_2$, and 2.5 units of taq DNA polymerase
(Roche Molecular Biochemicals). A GeneAmp PCR System
9700 (Applied Biosystems, Foster City, CA) was used to

25   run the following program: 94°C for 5 minutes; 40
cycles of [94°C for 45 seconds, 55°C for 45 seconds,
72°C for 90 seconds]; 72°C for 10 minutes; cool to 4°C.
The PCR products were purified with QIAquick 96 PCR
Purification Kit (Qiagen Inc.) according to the

30   manufacturer's directions. All purified PCR products
were sequenced with the primer 5'-

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CGGATAACAATTTCACACAGG-3' (SEQ ID NO:283), using an ABI
3770 Sequencer (Perkin Elmer) according to the
manufacturer's directions.

5   V.   Sequence Ranking. The peptide sequences that were
     translated from nucleotide sequences (Section IV.C.)
     were correlated to ELISA data. The peptides considered
     for modification were those expressed by phage clones
     that yielded higher OD_{450} readings in the NGF-coated
10   wells relative to the OD_{450} reading produced in the
     corresponding BSA-coated wells. The peptides encoded
     by sequences that occurred multiple times were also
     considered for modification. On these criteria, the
     top two (2) peptides from library 93, top nine (9)
15   peptides from library 72, top seventeen (17) peptides
     from library 39, top eleven (11) peptides from library
     95, top six (6) peptides from library 59, and top ten
     (10) peptides from library 63 were selected for
     modification.
20   The candidate peptides selected from the affinity
     matured population were used to construct fusion
     proteins in which a monomer of each peptide was fused
     in-frame to the Fc region of human IgG1. For fusion of
     peptides to the N-terminus of Fc, constructs were made
25   by annealing pairs of oligonucleotides ("oligos") to
     generate a duplex encoding the peptide and a linker
     comprised, depending on the peptide, of five glycine
     residues, one leucine residue and one glutamic acid
     residue as an *NdeI* to *XhoI* fragment. These duplex
30   molecules were ligated into a vector (pAMG21-Fc N-
     terminal, described above) containing the human Fc

gene, also digested with NdeI and XhoI. The resulting
ligation mixtures were transformed by electroporation
into E. coli strain 2596 cells (GM221, described
further below). Clones were screened for the ability

5   to produce the recombinant protein product and to
possess the gene fusion having a correct nucleotide
sequence. A single such clone was selected for each of
the modified peptides (i.e., Fc-peptide fusion
products) shown in Table 5. For fusion of peptides to

10   the C-terminus of Fc, constructs were made by annealing
pairs of oligonucleotides ("oligos") to generate a
duplex encoding five glycine residues, one alanine and
one glutamine residue, the peptide, followed by one
leucine residue and one glutamic acid residue as an

15   ApaLI to XhoI fragment. These duplex molecules were
ligated into a vector (pAMG21-Fc C-terminal, described
above) containing the human Fc gene, also digested with
ApaLI and XhoI. The resulting ligation mixtures were
transformed and screened as described above. Truncated

20   versions of select peptides were made by PCR, using the
parent construct as template. PCR products encoding
the desired sequence were ligated into the parent
construct as BsrGI to BamHI, NcoI to BsrGI or NdeI to
BsrGI fragments. The resulting ligation mixtures were

25   transformed and screened as described above.
       Results from testing of the resulting modified
peptides in the DRG neutralization assay (see above for
protocol) are given in Table 5. The effectiveness of
peptides selected from the affinity matured population

30   may be determined in the DRG neutralization assay in
the same manner.

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Table 5

In vitro NGF Inhibition Activity of Matured Modified Peptides

Linker (L) Portion of (L)-(P) fusion (SEQ ID NO:)	Peptide (P) Portion of (L)-(P) fusion (SEQ ID NO:)	Attachment of (L)-(P) fusion to Fc domain*	In Vivo anti-NGF Activity of Matured Modified Peptide
285	202	C-term	+
285	203		+
285	204		−
285	205	C-term	−
285	206	C-term	−
285	207		−
285	208		+
285	209		+
286	210	C-term	+
285	211	C-term	+
285	212	C-term	−
285	213	C-term	−
285	214		+
285	215		+
285	216	C-term	+
285	217		+
285	218		−
285	219	C-term	+
285	220		−
285	221	C-term	+
285	222		+
285	223		−
285	224	C-term	+
285	225	C-term	−
285	226	C-term	−
286	227	C-term	+
286	228		+
285	229		+
285	230	C-term	+
286	231	C-term	+
285	232	C-term	+
285	233		+
285	234		+
285	235	C-term	+
285	236	C-term	+

285	237	C-term	+
285	238	C-term	−
285	239	C-term	+
285	240		+
285	241	C-term	+
285	242		−
285	243		+
285	244		−
285	245		−
285	246		+
286	247		+
285	248		−
285	249		+
285	250	C-term	−
285	251	C-term	+
285	252		−
285	253	C-term	−
285	254		+
285	255		+
285	256		−
285	257		−
285	258	C-term	−
285	259	C-term	+
285	260	C-term	−
285	261		−
285	262	C-term	−
285	263		+
285	264		−
285	265	C-term	−
285	266		+
285	267		−
285	268		−
285	269		−
285	270		−
285	271	C-term	+
285	272	C-term	+
285	279	C-term	+
285	280	C-term	+

"+" indicates at least 50% inhibition of NGF induced activity observed at matured modified peptide concentrations of 20 nM or lower

"−" indicates less than 50% inhibition of NGF induced activity observed at matured modified peptide concentrations of at least 20 nM.

"n/a" means not applicable.

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*(L)-(P) fusions were attached to the N-terminus of
Fc domain (SEQ ID NO:60 lacking N-terminal methionine)
unless designated "C-term" wherein the (L)-(P) fusions
were attached to the C-terminus of Fc domain (SEQ ID
5   NO:60).

**Example 5: *In vivo* antinociceptive activity of
anti-NGF matured modified peptides in rat pain models**

10   A. Neuropathic Pain Model.   Male Sprague-Dawley
rats (200 g) were anesthetized with isoflurane inhalant
anesthesia and the left lumbar spinal nerves at the
level of L5 and L6 were tightly ligated (4-0 silk
suture) distal to the dorsal root ganglion and prior to
15   entrance into the sciatic nerve, as first described by
Kim and Chung (Kim, S.H.; Chung, J.M. An experimental
model for peripheral neuropathy produced by segmental
spinal nerve ligation in the rat. Pain 50:355-363,
(1992)). The incisions were closed and the rats were
20   allowed to recover. This procedure results in
mechanical (tactile) allodynia in the left hind paw as
assessed by recording the pressure at which the
affected paw (ipsilateral to the site of nerve injury)
was withdrawn from graded stimuli (von Frey filaments
25   ranging from 4.0 to 148.1 mN) applied perpendicularly
to the plantar surface of the paw (between the
footpads) through wire-mesh observation cages. A paw
withdrawal threshold (PWT) was determined by
sequentially increasing and decreasing the stimulus
30   strength and analyzing withdrawal data using a Dixon
non-parametric test, as described by Chaplan et al.
(Chaplan, S.R.; Bach, F.W.; Pogrel, J.W.; Chung, J.M.;
Yaksh, T.L. Quantitative assessment of tactile

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allodynia in the rat paw. J. Neurosci. Meth, 53:55-63
(1994)).

Normal rats and sham surgery rats (nerves isolated
but not ligated) withstand at least 148.1 mN
5   (equivalent to 15 g) of pressure without responding.
Spinal nerve ligated rats respond to as little as 4.0
mN (equivalent to 0.41 g) of pressure on the affected
paw. Rats were included in the study only if they did
not exhibit motor dysfunction (e.g., paw dragging or
10   dropping) and their PWT was below 39.2 mN (equivalent
to 4.0 g). At least seven days after surgery rats were
treated with a matured modified peptide previously
observed to inhibit at least 50% of NGF induced
activity *in vitro* at concentrations of 20 nM or lower
15   (matured modified peptides designated as "+" in
Table 5). Generally, rats were treated with a
screening dose of 60 mg/kg of the matured modified
peptide or control diluent (PBS) once by s.c. injection
and PWT was determined each day thereafter for 7 days
20   (Figure 5).

B.   CFA Inflammatory Pain Model. Male Sprague-
Dawley rats (200 g) were lightly anesthetized with
isoflurane inhalant anesthesia and the left hindpaw was
25   injected with complete Freund's adjuvant (CFA), 0.15
ml. This procedure results in mechanical (tactile)
allodynia in the left hind paw as assessed by recording
the pressure at which the affected paw was withdrawn
from graded stimuli (von Frey filaments ranging from
30   4.0 to 148.1 mN) applied perpendicularly to the plantar
surface of the paw (between the footpads) through wire-

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mesh observation cages. PWT was determined by
sequentially increasing and decreasing the stimulus
strength and analyzing withdrawal data using a Dixon
non-parametric test, as described by Chaplan et al.

5   (1994). Rats were included in the study only if they
did not exhibit motor dysfunction (e.g., paw dragging
or dropping) or broken skin and their PWT was below
39.2 mN (equivalent to 4.0 g).

At least seven days after CFA injection rats were

10   treated with a matured modified peptide previously
observed to inhibit at least 50% of NGF induced
activity in vitro at concentrations of 20 nM or lower
(matured modified peptides designated as "+" in
Table 5). Generally, rats were treated with a

15   screening dose of 60 mg/kg of the matured modified
peptide or control diluent (PBS) once by s.c. injection
and PWT was determined each day thereafter for 7 days.
Average paw withdrawal threshold (PWT) was converted to
percent of maximum possible effect (%MPE) using the

20   following formula: %MPE = 100 * (PWT of treated rats -
PWT of control rats)/(15-PWT of control rats). Thus,
the cutoff value of 15 g (148.1 mN) is equivalent to
100% of the MPE and the control response is equivalent
to 0% MPE.

25   At the screening dose of 60 mg/kg, certain
modified peptides produced an antinociceptive effect
within three or four days following a single s.c.
injection (Figure 6). Observable effects of active
modified peptides generally subsided between days five

30   and six following the injection.

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Table 6

In vivo antinociceptive activity of anti-NGF matured modified peptides in rat pain models

Linker (L) Portion of (L)-(P) fusion (SEQ ID NO:)	Peptide (P) Portion of (L)-(P) fusion (SEQ ID NO:)	Attachment of (L)-(P) Fusion to Fc domain*	*In Vivo* anti-NGF Activity of Matured Modified Peptide
285	219	C-term	Fig. 5, A
285	251	C-term	Fig. 5, B
285	236	C-term	Fig. 5, C
285	233		Fig. 5, D
285	246		Fig. 5, E
285	208		Fig. 5, F
285	224	C-term	Fig. 5, G
285	241	C-term	Fig. 5, H
285	239	C-term	Fig. 6, I
285	266		Fig. 6, J

*(L)-(P) fusions were attached to the N-terminus of Fc domain (SEQ ID NO:60 lacking N-terminal methionine) unless designated "C-term" wherein the (L)-(P) fusions were attached to the C-terminus of Fc domain (SEQ ID NO:60).

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CLAIMS

What is claimed is:

5   1.   A peptide comprising an amino acid sequence of
the formula:

$a^1a^2a^3Ca^5a^6a^7a^8a^9a^{10}a^{11}LQSCa^{16}a^{17}a^{18}$ (SEQ ID NO: 276)
wherein:

a^1, a^2, a^3, a^5, a^6, a^8, and a^{18} are each
10   independently absent or amino acid residues;

a^7 is a neutral hydrophobic or polar
hydrophobic amino acid residue;

a^9 is a neutral hydrophobic or polar
hydrophobic amino acid residue;

15   a^{10} is a neutral hydrophobic, neutral polar, or
a basic amino acid residue;

a^{11} is a neutral hydrophobic, neutral polar, or
a basic amino acid residue;

a^{16} is a neutral hydrophobic amino acid residue;

20   a^{17} is a neutral hydrophobic or polar
hydrophobic amino acid residue; or a physiologically
acceptable salt thereof, and wherein said peptide is
capable of modulating NGF activity.

25   2.   The peptide according to Claim 1 wherein:

a^1 is L, P, W, F, S, Y, N, R, or H;

a^2 is L, W, F, S, Y, D, or R;

a^3 is L, I, W, Y, D, or E;

a^5 is A, L, G, D, E, or K;

30   a^6 is A, L, F, S, N, E, K, or H;

a^7 is L, P, Y, or N;

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a^8 is A, P, M, F, G, Q, or D;

a^9 is P, M, T, G, or S;

a^{10} is A, V, F, H, G, or S;

a^{11} is V, L, I, F, T, N, or K;

5   a^{16} is L, I, W, M, or F;

a^{17} is A, G, S, W, or N;

a^{18} is F, W, or Y.

3.   The peptide according to Claim 2 wherein:

10   a^1 is W;

a^2 is D;

a^6 is F;

a^{10} is A;

a^{11} is K;

15   a^{16} is F.

4.   The peptide according to Claim 3 wherein a^1-a^{18}
is WDMCHFSHAAKLQSCFPH (SEQ ID NO:273).

20   5.   A modified peptide comprising at least one
peptide according to Claims 1-4 and at least one
vehicle, wherein said modified peptide is capable of
modulating NGF activity.

25   6.   The modified peptide according to Claim 5
wherein said vehicle is selected from the group
consisting of a Fc domain, polyethylene glycol, a
lipid, a cholesterol group, a carbohydrate, and an
oligosaccharide.

30

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7. The peptide according to Claim 1, 2, 3, or 4
which is cyclic.

8. A dimer or multimer of the peptide according to
Claim 1, 2, 3, or 4.

9. A modified peptide having the formula:
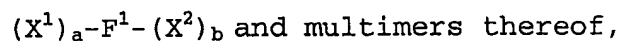
$(X^1)_a-F^1-(X^2)_b$ and multimers thereof,

wherein:

F^1 is a vehicle;

X^1 and X^2 are each independently selected from

$-(L^1)_c-P^1$;

$-(L^1)_c-P^1-(L^2)_d-P^2$;

$-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$, and

$-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$;

wherein one or more of P^1, P^2, P^3, and P^4 each
independently comprise a peptide according to Claim 1,
2, 3, or 4;

L^1, L^2, L^3, and L^4 are each independently linkers;
and

a, b, c, d, e, and f are each independently 0 or
1, provided that at least one of a and b is 1; or a
physiologically acceptable salt thereof, and wherein
said peptide is capable of modulating NGF activity.

10. The modified peptide according to Claim 9
wherein one or more of P^1, P^2, P^3, and P^4 each
independently comprise a peptide as shown in SEQ ID
NO:273.

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11.   The modified peptide of Claim 9 of the
formula: X^1-F^1 or F^1-X^2 or a physiologically acceptable
salt thereof.

5   12.   The modified peptide of Claim 9 of the
formula: F^1-$(L^1)_c$-P^1 or a physiologically acceptable
salt thereof.

13.   The modified peptide of Claim 9 of the
10   formula: F^1-$(L^1)_c$-P^1-$(L^2)_d$-P^2 or a physiologically
acceptable salt thereof.

14.   The modified peptide of Claim 9 of the
formula: P^1-$(L^1)_c$-F^1-$(L^2)_d$-P^2 or a physiologically
15   acceptable salt thereof.

15.   The modified peptide of Claim 9, 10, 11, 12,
13, or 14 wherein F^1 is an Fc domain or fragment
thereof.
20

16.   The modified peptide of Claim 15 wherein F^1
comprises the amino acid sequence of SEQ ID NO: 60.

17.   A polynucleotide encoding the peptide or
25   modified peptide according to Claim 1, 5, 9, 14, or 16.

18.   An expression vector comprising the
polynucleotide of Claim 17.

30   19.   A host cell comprising the expression vector
of Claim 18.

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20. The host cell according to Claim 19 wherein the cell is a prokaryotic cell.

5   21. The host cell according to Claim 20 wherein the cell is an *E. coli* cell.

22. The host cell according to Claim 19 wherein the cell is a eukaryotic cell.

10

23. A peptide comprising an amino acid sequence of the formula:

$b^1b^2b^3CWb^6b^7b^8b^9GCb^{12}b^{13}b^{14}$ (SEQ ID NO: 274)

wherein:

15   b^1, b^2, b^3, b^8, b^9, b^{13} and b^{14} are each independently absent or amino acid residues;

b^6 is a neutral hydrophobic amino acid residue;

b^7 is a polar hydrophobic amino acid residue;

b^{12} is a neutral hydrophobic or an acidic amino

20 acid residue; or a physiologically acceptable salt thereof, and wherein said peptide is capable of modulating NGF activity.

24. The peptide according to Claim 23 wherein:

25   b^1 is V, L, I, W, T, Y, or E;

b^2 is L, W, M, Q, or H;

b^3 is W, M, G, Q, or E;

b^6 is F or W;

b^7 is T or S;

30   b^8 is A, P, W, S, E, or D;

b^9 is A, G, Q, E, or K;

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b^{12} is V, I, P, D, or E.

25. The peptide according to Claim 23 wherein:

b^1 is L or I;

5   b^2 is Q or H;

b^3 is G or M;

b^6 is F or W;

b^7 is T or S;

b^8 is E or D;

10   b^9 is E or K;

b^{12} is V or E;

b^{13} is V, W, or Y;

b^{14} is P, S, or Q.

15   26. A peptide selected from the group consisting
of IHGCWFTEEGCVWQ (SEQ ID NO: 277) and LQMCWFTEKGCEVP
(SEQ ID NO: 278), or a physiologically acceptable salt
thereof, wherein said peptide is capable of modulating
NGF activity.

20

        27. A composition of matter comprising at least
one peptide according to Claim 23, 24, or 25 and at
least one vehicle, wherein said composition of matter
is capable of modulating NGF activity.

25

        28. The composition of matter according to Claim
27 wherein said vehicle is selected from the group
consisting of a Fc domain, polyethylene glycol, a
lipid, a cholestérol group, a carbohydrate, and an
30 oligosaccharide.

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29. The peptide according to Claim 23, 24, or 25 which is cyclic.

30. A dimer or multimer of the peptide according to Claim 23, 24, or 25.

31. A composition of matter having the formula:

$(X^1)_a-F^1-(X^2)_b$ and multimers thereof,

wherein:

F^1 is a vehicle;

X^1 and X^2 are each independently selected from

$-(L^1)_c-P^1$;

$-(L^1)_c-P^1-(L^2)_d-P^2$;

$-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$; and

$-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$;

wherein one or more of P^1, P^2, P^3, and P^4 each independently comprise a peptide according to Claim 23, 24, or 25;

L^1, L^2, L^3, and L^4 are each independently linkers; and a, b, c, d, e, and f are each independently 0 or 1, provided that at least one of a and b is 1; or a physiologically acceptable salt thereof, and wherein said peptide is capable of modulating NGF activity.

32. The composition of matter of Claim 31 wherein one or more of P^1, P^2, P^3, and P^4 each independently comprise a peptide selected from the group consisting of SEQ ID NOS: 277 and 278.

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33. The composition of matter of Claim 31 of the formula: X^1-F^1 or F^1-X^2 or a physiologically acceptable salt thereof.

5   34. The composition of matter of Claim 31 of the formula: $F^1-(L^1)_c-P^1$ or a physiologically acceptable salt thereof.

35. The composition of matter of Claim 31 of the

10   formula: $F^1-(L^1)_c-P^1-(L^2)_d-P^2$ or a physiologically acceptable salt thereof.

36. The composition of matter of Claim 31 of the formula: $P^1-(L^1)_c-F^1-(L^2)_d-P^2$ or a physiologically

15   acceptable salt thereof.

37. The composition of matter of Claim 31, wherein F^1 is an Fc domain or fragment thereof.

20   38. The composition of matter of Claim 31 wherein F^1 comprises the amino acid sequence of SEQ ID NO: 60 or a fragment thereof.

39. A polynucleotide encoding the peptide or

25   polypeptide according to Claim 23, 27, 31, 36, or 38.

40. An expression vector comprising the polynucleotide of Claim 39.

30   41. A host cell comprising the expression vector of Claim 40.

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42. The host cell according to Claim 41 wherein
the cell is a prokaryotic cell.

5   43. The host cell according to Claim 42 wherein
the cell is an *E. coli* cell.

43. The host cell according to Claim 41 wherein
the cell is a eukaryotic cell.

10

45. A peptide comprising an amino acid sequence
of the formula:
$$c^1c^2QCc^5c^6Sc^8c^9GCc^{12}c^{13}c^{14}c^{15}c^{16}$$
wherein:

15   c^1, c^5, c^8, c^9, c^{13} and c^{14} are each independently
absent or amino acid residues;
       c^2 is a neutral hydrophobic amino acid residue;
       c^6 is a neutral hydrophobic or polar hydrophopic
amino acid residue;
20   c^{12} is a neutral hydrophobic or an acidic amino
acid residue; or a physiologically acceptable salt
thereof, and wherein said peptide is capable of
modulating NGF activity.

25   46. The peptide according to Claim 45 wherein:
       c^1 is V, I, T, Y, N, or K;
       c^2 is L, M, or F;
       c^5 is S, Q, or E;
       c^6 is L, F, W, or Y;
30   c^8 is W, M, T, G, S, or N;
       c^9 is A, V, G, S, or E;

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c^{12} is L, P, G, D, or E;

c^{13} is K, I, L, Y, or Q;

c^{14} is A, S, P, V, M, or Q.

5   47.   The peptide according to Claim 46 wherein:

c^{1} is K or T;

c^{2} is L or F;

c^{5} is E or Q;

c^{6} is L, F, or Y;

10   c^{8} is T;

c^{9} is S or A;

c^{12} is P or L;

c^{15} is L or absent;

c^{16} is E or absent.

15

48.   A peptide selected from the group consisting
of SEQ ID NOS: 208, 209, 224, 233, 234, 241, 246, 279,
and 280, or a physiologically acceptable salt thereof,
wherein said peptide is capable of modulating NGF

20   activity.

49.   A composition of matter comprising at least
one peptide according to Claim 45, 46, 47, or 48 and at
least one vehicle, wherein said composition of matter

25   is capable of modulating NGF activity.

50.   The composition of matter according to Claim
49 wherein said vehicle is selected from the group
consisting of a Fc domain, polyethylene glycol, a

30   lipid, a cholesterol group, a carbohydrate, and an
oligosaccharide.

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51. The peptide according to Claim 45, 46, 47, or 48 which is cyclic.

5   52. A dimer or multimer of the peptide according to Claim 45, 46, 47, or 48.

53. A composition of matter having the formula:

$(X^1)_a-F^1-(X^2)_b$ and multimers thereof,

10   wherein:

F^1 is a vehicle;

X^1 and X^2 are each independently selected from

$-(L^1)_c-P^1$;

$-(L^1)_c-P^1-(L^2)_d-P^2$;

15        $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$; and

$-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$;

wherein one or more of P^1, P^2, P^3, and P^4 each independently comprise a peptide according to Claim 45, 46, 47, or 48;

20   L^1, L^2, L^3, and L^4 are each independently linkers; and a, b, c, d, e, and f are each independently 0 or 1, provided that at least one of a and b is 1; or a physiologically acceptable salt thereof, and wherein said peptide is capable of modulating NGF activity.

25

54. The composition of matter of claim 53 wherein one or more of P^1, P^2, P^3, and P^4 each independently comprise a peptide selected from the group consisting of SEQ ID NOS: 208, 209, 224, 233, 234, 241, 246, 279,

30   and 280.

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55. The composition of matter of Claim 53 of the
formula: X^1-F^1 or F^1-X^2 or a physiologically acceptable
salt thereof.

5   56. The composition of matter of Claim 53 of the
formula: F^1-$(L^1)_c$-P^1 or a physiologically acceptable
salt thereof.

57. The composition of matter of Claim 53 of the
10 formula: F^1-$(L^1)_c$-P^1-$(L^2)_d$-P^2 or a physiologically
acceptable salt thereof.

58. The composition of matter of Claim 53 of the
formula: P^1-$(L^1)_c$-F^1-$(L^2)_d$-P^2 or a physiologically
15 acceptable salt thereof.

59. The composition of matter of Claim 53,
wherein F^1 is an Fc domain or fragment thereof.

20   60. The composition of matter of Claim 53 wherein
F^1 comprises the amino acid sequence of SEQ ID NO: 60
or a fragment thereof.

61. A polynucleotide encoding the peptide or
25 polypeptide according to Claim 45, 47, 49, 53, or 56.

62. An expression vector comprising the
polynucleotide of Claim 61.

30   63. A host cell comprising the expression vector
of Claim 62.

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64. The host cell according to Claim 63 wherein the cell is a prokaryotic cell.

5   65. The host cell according to Claim 64 wherein the cell is an *E. coli* cell.

66. The host cell according to Claim 63 wherein the cell is a eukaryotic cell.

10

67. A peptide comprising an amino acid sequence of the formula:

$$d^1 d^2 d^3 d^4 d^5 d^6 d^7 PP d^{10} d^{11} d^{12} d^{13} d^{14} d^{15} P d^{17} d^{18} d^{19} d^{20} d^{21} d^{22} d^{23} d^{24}$$

wherein:

15   d^1 is a W, Y, Q, or E;

d^2 is a V, L, F, S, or Q;

d^3 is a W, F, G, S, or Q;

d^4 is a A, Q, D, E, or K;

d^5 is a V, W, G, or R;

20   d^6 is a M, S, Y, Q, N, E, K, or R;

d^7 is a A, V, L, P, W, Q, or H;

d^{10} is a D or E;

d^{11} is a V or I;

d^{12} is a V, L, F, or Y;

25   d^{13} is a V, L, G, Q, or E;

d^{14} is a Q, D, or E;

d^{15} is a W or C;

d^{17} is a W, Y, or Q;

d^{18} is a V, T, Q, N, or K;

30   d^{19} is a A, L, or P;

d^{20} is a P, Q, R, or H;

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d^{21} is a V, I, W, D;

d^{22} is a A, I, S, Q, or D;

d^{23} is a L or absent;

d^{24} is a E or absent;

5   or a physiologically acceptable salt thereof, and
wherein said peptide is capable of modulating NGF
activity.

         68.   The peptide according to Claim 67 wherein:

10   d^{1} is Q or Y;

             d^{2} is L;

             d^{3} is W or D;

             d^{4} is D, E, or K;

             d^{5} is V, W, or G;

15   d^{6} is Q, K, or R;

             d^{7} is W or L;

             d^{10} is E or D;

             d^{11} is V or I;

             d^{12} is V, L, or F;

20   d^{13} is V, L, or G;

             d^{14} is Q, D, or E;

             d^{15} is W or C;

             d^{17} is W or Y;

             d^{18} is Q, K, or N;

25   d^{19} is P, V, or L;

             d^{20} is P or S;

             d^{21} is V;

             d^{22} is Q or D;

             d^{23} is a L or absent;

30   d^{24} is a E or absent.

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    69. A peptide selected from the group consisting
of SEQ ID NOS: 203, 228, 240, 247, and 266, inclusive,
or a physiologically acceptable salt thereof, wherein
said peptide is capable of modulating NGF activity.

5

    70. A composition of matter comprising at least
one peptide according to Claim 67, 68, or 69 and at
least one vehicle, wherein said composition of matter
is capable of modulating NGF activity.

10

    71. The composition of matter according to Claim
70 wherein said vehicle is selected from the group
consisting of a Fc domain, polyethylene glycol, a
lipid, a cholesterol group, a carbohydrate, and an

15 oligosaccharide.

    72. The peptide according to Claim 67, 68, or 69
which is cyclic.

20   73. A dimer or multimer of the peptide according
to Claim 67, 68, or 69.

    74. A composition of matter having the formula:
        $(X^1)_a-F^1-(X^2)_b$ and multimers thereof,

25 wherein:
        F^1 is a vehicle;
        X^1 and X^2 are each independently selected from
            $-(L^1)_c-P^1$;
            $-(L^1)_c-P^1-(L^2)_d-P^2$;

30          $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$; and
            $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$;

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wherein one or more of P^1, P^2, P^3, and P^4 each
independently comprise a peptide according to Claim 67,
68, or 69;

L^1, L^2, L^3, and L^4 are each independently linkers;

5   and a, b, c, d, e, and f are each independently 0 or 1,
provided that at least one of a and b is 1; or a
physiologically acceptable salt thereof, and wherein
said peptide is capable of modulating NGF activity.

10   75.   The composition of matter of Claim 73 wherein
one or more of P^1, P^2, P^3, and P^4 each independently
comprise a peptide selected from the group consisting
of SEQ ID NOS: 203, 228, 240, 247, and 266.

15   76.   The composition of matter of Claim 73 of the
formula: X^1-F^1 or F^1-X^2 or a physiologically acceptable
salt thereof.

          77.   The composition of matter of Claim 73 of the
20   formula: F^1-$(L^1)_c$-P^1 or a physiologically acceptable
salt thereof.

          78.   The composition of matter of Claim 73 of the
formula: F^1-$(L^1)_c$-P^1-$(L^2)_d$-P^2 or a physiologically
25   acceptable salt thereof.

          79.   The composition of matter of Claim 73 of the
formula: P^1-$(L^1)_c$-F^1-$(L^2)_d$-P^2, or a physiologically
acceptable salt thereof.

30   80.   The composition of matter of Claim 73,
wherein F^1 is an Fc domain or fragment thereof.

81. The composition of matter of Claim 73 wherein F^1 comprises the amino acid sequence of SEQ ID NO: 60 or fragments thereof.

82. A polynucleotide encoding the peptide or polypeptide according to Claim 67, 68, or 69.

83. An expression vector comprising the polynucleotide of Claim 82.

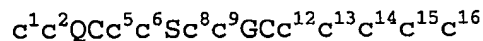84. A host cell comprising the expression vector of Claim 83.

85. The host cell according to Claim 84 wherein the cell is a prokaryotic cell.

86. The host cell according to Claim 85 wherein the cell is an *E. coli* cell.

87. The host cell according to Claim 84 wherein the cell is a eukaryotic cell.

88. A peptide comprising an amino acid sequence of the formula:
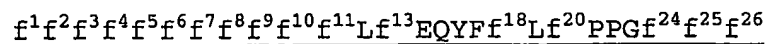
$$f^1f^2f^3f^4f^5f^6f^7f^8f^9f^{10}f^{11}Lf^{13}EQYFf^{18}Lf^{20}PPGf^{24}f^{25}f^{26}$$

wherein:

f^1-f^6, f^8, f^9, f^{11}, f^{18}, f^{24}, f^{25} and f^{26} are each independently absent or amino acid residues;

f^7, f^{10}, and f^{13} are each independently neutral hydrophobic or polar hydrophopic amino acid residues;

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f^{20} is a T, M, or I;

or a physiologically acceptable salt thereof, and
wherein said peptide is capable of modulating NGF
activity.

5

89.   The peptide according to Claim 88 wherein:

f^1 is A or absent;

f^2 is Q or absent;

f^3 is L, M, T, Q or N;

10   f^4 is A, I, P, T, G, or Q;

f^5 is M, G, E, or D;

f^6 is W or H;

f^7 is L, P, or M;

f^8 is G, L, R, or S;

15   f^9 is A, Q, D, or E;

f^{10} is L, N, or M;

f^{11} is P, R, or S;

f^{13} is L, F, or Y;

f^{18} is A, Q, E, or R;

20   f^{20} is T, M, or I;

f^{24} is L, I, V, or Y;

f^{25} is a L or absent;

f^{26} is a E or absent.

25   90.   The peptide according to Claim 89 wherein:

f^1 is A or absent;

f^2 is Q or absent;

f^3 is M, Q, or N;

f^4 is I, P, T, or G;

30   f^5 is M, G, or D;

f^6 is W or H;

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f^7 is L or P;

f^8 is G, L, or S;

f^9 is A, Q, or D;

f^{10} is L, N, or M;

5   f^{11} is P, R, or S;

f^{13} is L or F;

f^{18} is A, Q, or E;

f^{20} is T, M, or I;

f^{24} is L, I, or V;

10   f^{25} is a L or absent;

f^{26} is a E or absent.

91.   A peptide selected from the group consisting
of SEQ ID NOS: 210, 230, 232, 236, 239, and 251,
15 inclusive, or a physiologically acceptable salt
thereof, and wherein said peptide is capable of
modulating NGF activity.

92.   A modified peptide comprising at least one
peptide according to Claim 88, 89, 90, or 91 and at
20 least one vehicle, and wherein said peptide is capable
of modulating NGF activity.

93.   The composition of matter according to Claim
92 wherein said vehicle is selected from the group
25 consisting of a Fc domain, polyethylene glycol, a
lipid, a cholesterol group, a carbohydrate, and an
oligosaccharide.

94.   The peptide according to Claim 88, 89, 90, or
30 91 which is cyclic.

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95. A dimer or multimer of the peptide according
to Claim 88, 89, 90, or 91.

96. A composition of matter having the formula:

5          $(X^1)_a$-F^1-$(X^2)_b$ and multimers thereof,
wherein:
    F^1 is a vehicle;
    X^1 and X^2 are each independently selected from
       $-(L^1)_c$-P^1;
10      $-(L^1)_c$-P^1-$(L^2)_d$-P^2;
       $-(L^1)_c$-P^1-$(L^2)_d$-P^2-$(L^3)_e$-P^3; and
       $-(L^1)_c$-P^1-$(L^2)_d$-P^2-$(L^3)_e$-P^3-$(L^4)_f$-P^4;
wherein one or more of P^1, P^2, P^3, and P^4 each
independently comprise a peptide according to Claim 88,
15 89, 90, or 91;
    L^1, L^2, L^3, and L^4 are each independently linkers;
and a, b, c, d, e, and f are each independently 0 or 1,
provided that at least one of a and b is 1; or a
physiologically acceptable salt thereof, and wherein
20 said peptide is capable of modulating NGF activity.

97. The composition of matter of claim 96 wherein
one or more of P^1, P^2, P^3, and P^4 each independently
comprise a peptide selected from the group consisting
25 of SEQ ID NOS: 210, 230, 232, 236, 239, and 251.

98. The composition of matter of Claim 96 of the
formula: X^1-F^1 or F^1-X^2 or a physiologically acceptable
salt thereof.

30

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99. The composition of matter of Claim 96 of the
formula: $F^1-(L^1)_c-P^1$ or a physiologically acceptable
salt thereof.

5   100. The composition of matter of Claim 96 of the
formula: $F^1-(L^1)_c-P^1-(L^2)_d-P^2$ or a physiologically
acceptable salt thereof.
         101. The composition of matter of Claim 96 of the
formula: $P^1-(L^1)_c-F^1-(L^2)_d-P^2$ or a physiologically
10 acceptable salt thereof.

         102. The composition of matter of Claim 96
wherein F^1 is an Fc domain or fragment thereof.

15   103. The composition of matter of Claim 96
wherein F^1 comprises the amino acid sequence of SEQ ID
NO: 60 or fragments thereof.

         104. A polynucleotide encoding the peptide or
20 polypeptide according to Claim 88, 89, 90, or 91.

         105. An expression vector comprising the
polynucleotide of Claim 104.

25   106. A host cell comprising the expression vector
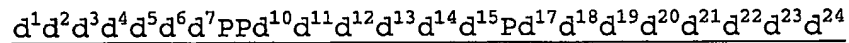of Claim 105.

         107. The host cell according to Claim 106 wherein
the cell is a prokaryotic cell.
30

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108. The host cell according to Claim 107 wherein the cell is an *E. coli* cell.

109. The host cell according to Claim 106 wherein
5   the cell is a eukaryotic cell.

110. A peptide comprising an amino acid sequence of the formula:

$$h^1h^2h^3h^4h^5h^6LGh^9h^{10}h^{11}Lh^{13}YFh^{16}Lh^{18}PPGh^{22}h^{23}h^{24}$$
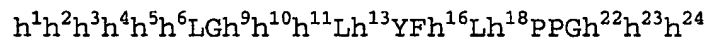
10   wherein:
       h^1-h^6, h^9, h^{11}, h^{23}, and h^{24} are each independently absent or amino acid residues;
       h^{10} and h^{13} are each independently neutral hydrophobic or polar hydrophopic amino acid residues;
15   h^{16} is a polar hydrophopic or basic amino acid residue;
       h^{18} is a neutral hydrophopic amino acid residue;
       h^{22} is a neutral polar amino acid residue;
or a physiologically acceptable salt thereof, and
20   wherein said peptide is capable of modulating NGF activity.

111. The peptide according to Claim 110 wherein:
       h^1 is A or absent;
25   h^2 is Q or absent;
       h^3 is V, G, P, or absent;
       h^4 is V, T, S, K, or absent;
       h^5 is S, E, Q, or D;
       h^6 is Q, N, K, or M;
30   h^9 is S, G, Q, or D;
       h^{10} is W, Y, or F;

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h^{11} is A, L, or M;

h^{13} is Q, N, or Y;

h^{16} is K, H, S, or R;

h^{18} is A, V, L, or I;

5   h^{22} is S, T, or G;

h^{23} is L or absent;

h^{24} is E or absent.

112.   The peptide according to Claim 111 wherein:

10   h^1 is A or absent;

h^2 is Q or absent;

h^3 is P or absent;

h^4 is T or absent;

h^5 is E or D;

15   h^6 is Q or N;

h^9 is E or D;

h^{10} is W or Y;

h^{11} is L or M;

h^{13} is Q or N;

20   h^{16} is K or R;

h^{18} is V or L;

h^{22} is S or T;

h^{23} is L or absent;

h^{24} is E or absent.

25

113.   A peptide selected from the group consisting
of SEQ ID NOS: 202, 211, 219, 221, 231, 237, and 272,
inclusive, or a physiologically acceptable salt
thereof, and wherein said peptide is capable of
30   modulating NGF activity.

114. A composition of matter comprising at least
one peptide according to Claim 110, 111, 112, or 113
and at least one vehicle, and wherein said peptide is
capable of modulating NGF activity.

5

115. The composition of matter according to Claim
114 wherein said vehicle is selected from the group
consisting of a Fc domain, polyethylene glycol, a
lipid, a cholesterol group, a carbohydrate, and an

10   oligosaccharide.

116. The peptide according to Claim 110, 111,
112, or 113 which is cyclic.

15   117. A dimer or multimer of the peptide according
to Claim 110, 111, 112, or 113.

118. A composition of matter having the formula:
$(X^1)_a-F^1-(X^2)_b$ and multimers thereof,

20   wherein:
       F^1 is a vehicle;
       X^1 and X^2 are each independently selected from
           $-(L^1)_c-P^1$;
           $-(L^1)_c-P^1-(L^2)_d-P^2$;

25         $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$; and
           $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$;
       wherein one or more of P^1, P^2, P^3, and P^4 each
       independently comprise a peptide according to Claim
       110, 111, 112, or 113;

30   L^1, L^2, L^3, and L^4 are each independently linkers;
       and a, b, c, d, e, and f are each independently 0 or 1,

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provided that at least one of a and b is 1; or a
physiologically acceptable salt thereof, and wherein
said peptide is capable of modulating NGF activity.

5   119. The composition of matter of Claim 118
wherein one or more of P^1, P^2, P^3, and P^4 each
independently comprise a peptide selected from the
group consisting of SEQ ID NOS: 202, 211, 219, 221,
231, 237, and 272.
10

           120. The composition of matter of Claim 118 of
the formula: X^1-F^1 or F^1-X^2 or a physiologically
acceptable salt thereof.

15   121. The composition of matter of Claim 118 of
the formula: F^1-$(L^1)_c$-P^1 or a physiologically
acceptable salt thereof.

           122. The composition of matter of Claim 118 of
20 the formula: F^1-$(L^1)_c$-P^1-$(L^2)_d$-P^2 or a physiologically
acceptable salt thereof.
           123. The composition of matter of Claim 118 of
the formula: P^1-$(L^1)_c$-F^1-$(L^2)_d$-P^2 or a physiologically
acceptable salt thereof.
25

           124. The composition of matter of Claim 118
wherein F^1 is an Fc domain or fragment thereof.

           125. The composition of matter of Claim 118
30 wherein F^1 comprises the amino acid sequence of SEQ ID
NO: 60 or fragments thereof.

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126.   A polynucleotide encoding the peptide or
polypeptide according to Claim 110, 111, 112, or 113.

5   127.   An expression vector comprising the
polynucleotide of Claim 126.

128.   A host cell comprising the expression vector
of Claim 127.

10

129.   The host cell according to Claim 128 wherein
the cell is a prokaryotic cell.

130.   The host cell according to Claim 129 wherein
15   the cell is an *E. coli* cell.

131.   The host cell according to Claim 128 wherein
the cell is a eukaryotic cell.

20   132.   A composition of matter comprising an amino
acid sequence of the formula: $(X^1)_a$-F^1-$(X^2)_b$
wherein:
     F^1 is an Fc domain;
     X^1 and X^2 are each independently selected from
25   -$(L^1)_c$-P^1, -$(L^1)_c$-P^1-$(L^2)_d$-P^2, -$(L^1)_c$-P^1-$(L^2)_d$-P^2-$(L^3)_e$-P^3,
and -$(L^1)_c$-P^1-$(L^2)_d$-P^2-$(L^3)_e$-P^3-$(L^4)_f$-P^4;
     L^1, L^2, L^3, and L^4 are each independently linkers;
     a, b, c, d, e, and f are each independently 0 or
1, provided that at least one of a and b is 1; and
30   P^1, P^2, P^3, and P^4 are each independently sequences
selected from the group consisting of:

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    i. SEQ ID NO: 1 to SEQ ID NO: 58, inclusive;

    ii. SEQ ID NO: 202 to SEQ ID NO: 280,
inclusive;

        iii. an analog of (i) or (ii); and

        iv. a derivative of (i), (ii) or (iii), and
wherein said composition of matter is capable of
modulating NGF activity.

    133.   The composition of matter of Claim 132
comprising an amino acid sequence of the formula:

                    F-L-P
wherein:

    F is an Fc domain;

    L is a linker; and

    P is selected from the group consisting of:

        i. SEQ ID NOS: 8, 10, 23, and 24;

        ii. an analog of (i); and

        iii. a derivative of (i) or (ii), and wherein
said composition of matter is capable of modulating NGF
activity.

    134.   A method of treating or preventing a disease
or disorder associated with NGF activity comprising
administering, to a human or animal subject, a
therapeutically effective amount of the composition
according to Claim 1, 5, 9, 14, 16, 23, 27, 31, 36, 38,
45, 48, 49, 51, 53, 56, 58, 67, 68, 70, 72, 74, 77, 79,
88, 89, 90, 92, 94, 96, 97, 99, 101, 110, 111, 112,
114, 116, 117, 118, 121, 123, 132, or 133.

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135.   The method according to Claim 134 wherein
the disease or disorder is selected from the group
consisting of neurologic pain, painful diabetic
neuropathy, post-herpetic neuralgia, inflammatory pain,

5   migraine, asthma, hyperactive bladder, psoriasis and
cancer.

136.   The method of Claim 134 wherein the disease
or disorder is pain.

10

137.   A method for modulating pain or promoting
analgesia comprising administering, to a human or
animal subject, a therapeutically effective amount of
the composition according to Claim 1, 5, 9, 14, 16, 23,

15   27, 31, 36, 38, 45, 48, 49, 51, 53, 56, 58, 67, 68, 70,
72, 74, 77, 79, 88, 89, 90, 92, 94, 96, 97, 99, 101,
110, 111, 112, 114, 116, 117, 118, 121, 123, 132, or
133.

20   138.   The method according to Claim 134 wherein
the disease or disorder is selected from the group
consisting of acute pain, dental pain, pain from
trauma, surgical pain, pain resulting from amputation
or abscess, causalgia, demyelinating diseases,

25   trigeminal neuralgia, cancer, chronic alcoholism,
stroke, thalamic pain syndrome, diabetes, acquired
immune deficiency syndrome ("AIDS"), toxins and
chemotherapy, general headache, migraine, cluster
headache, mixed-vascular and non-vascular syndromes,

30   tension headache, general inflammation, arthritis,
rheumatic diseases, lupus, osteoarthritis, inflammatory

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bowel disorders, inflammatory eye disorders,
inflammatory or unstable bladder disorders, psoriasis,
skin complaints with inflammatory components, sunburn,
carditis, dermatitis, myositis, neuritis, collagen
5   vascular diseases, chronic inflammatory conditions,
inflammatory pain and associated hyperalgesia and
allodynia, neuropathic pain and associated hyperalgesia
and allodynia, diabetic neuropathy pain, causalgia,
sympathetically maintained pain, deafferentation
10   syndromes, asthma, epithelial tissue damage or
dysfunction, herpes simplex, post-herpetic neuralgia,
disturbances of visceral motility at respiratory,
genitourinary, gastrointestinal or vascular regions,
wounds, burns, allergic skin reactions, pruritis,
15   vitiligo, general gastrointestinal disorders, colitis,
gastric ulceration, duodenal ulcers, vasomotor or
allergic rhinitis, or bronchial disorders, comprising
the step of administering a compound according to Claim
1, 5, 9, 14, 16, 23, 27, 31, 36, 38, 45, 48, 49, 51,
20   53, 56, 58, 67, 68, 70, 72, 74, 77, 79, 88, 89, 90, 92,
94, 96, 97, 99, 101, 110, 111, 112, 114, 116, 117, 118,
121, 123, 132, or 133.

     139. A pharmaceutical composition comprising a
25   compound according to Claim 1, 5, 9, 14, 16, 23, 27,
31, 36, 38, 45, 48, 49, 51, 53, 56, 58, 67, 68, 70, 72,
74, 77, 79, 88, 89, 90, 92, 94, 96, 97, 99, 101, 110,
111, 112, 114, 116, 117, 118, 121, 123, 132, or 133 and
a pharmaceutically-acceptable diluent or carrier.

30

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140.   The use of a compound according to Claim 1,
5, 9, 14, 16, 23, 27, 31, 36, 38, 45, 48, 49, 51, 53,
56, 58, 67, 68, 70, 72, 74, 77, 79, 88, 89, 90, 92, 94,
96,, 97, 99, 101, 110, 111, 112, 114, 116, 117, 118,

5   121, 123, 132, or 133 in the manufacture of a
medicament for the treatment of a disease or disorder
selected from the group consisting of acute pain,
inflammatory pain, neuropathic pain, dental pain, pain
from trauma, surgical pain, pain from amputation or

10   abscess, causalgia, demyelinating diseases, trigeminal
neuralgia, cancer, chronic alcoholism, stroke, thalamic
pain syndrome, diabetes, acquired immune deficiency
syndrome ("AIDS"), toxins and chemotherapy, general
headache, migraine, cluster headache, mixed-vascular

15   and non-vascular syndromes, tension headache, general
inflammation, arthritis, rheumatic diseases, lupus,
osteoarthritis, inflammatory bowel disorders,
inflammatory eye disorders, inflammatory or unstable
bladder disorders, psoriasis, skin complaints with

20   inflammatory components, sunburn, carditis, dermatitis,
myositis, neuritis, collagen vascular diseases, chronic
inflammatory conditions, inflammatory pain and
associated hyperalgesia and allodynia, neuropathic pain
and associated hyperalgesia and allodynia, diabetic

25   neuropathy pain, causalgia, sympathetically maintained
pain, deafferentation syndromes, asthma, epithelial
tissue damage or dysfunction, herpes simplex, post-
herpetic neuralgia, disturbances of visceral motility
at respiratory, genitourinary, gastrointestinal or

30   vascular regions, wounds, burns, allergic skin
reactions, pruritis, vitiligo, general gastrointestinal

137

disorders, colitis, gastric ulceration, duodenal
ulcers, vasomotor or allergic rhinitis, and bronchial
disorders.

5   141. A peptide comprising an amino acid sequence
selected from the group consisting of:

        i. SEQ ID NOS: 8, 10, 23, or 24;

        ii. an analog of (i); and

        iii. a physiologically acceptable salt of (i) or

10 (ii), wherein said peptide is capable of modulating NGF
activity.

        142. A composition of matter comprising at least
one peptide according to Claim 141 and at least one

15 vehicle, wherein said composition of matter is capable
of modulating NGF activity.

        143. The composition of matter according to Claim
142 wherein said vehicle is selected from the group

20 consisting of a Fc domain, polyethylene glycol, a
lipid, a cholesterol group, a carbohydrate, and an
oligosaccharide.

        144. The peptide according to Claim 141 which is

25 cyclic.

        145. A dimer or multimer of the peptide according
to Claim 141.

30   146. A composition of matter having the formula:
        $(X^1)_a-F^1-(X^2)_b$ and multimers thereof,

wherein:

F^1 is a vehicle;

X^1 and X^2 are each independently selected from

$-(L^1)_c-P^1$;

$-(L^1)_c-P^1-(L^2)_d-P^2$;

$-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$; and

$-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$;

wherein one or more of P^1, P^2, P^3, and P^4 each
independently comprise a peptide according to Claim
141;

L^1, L^2, L^3, and L^4 are each independently linkers;
and a, b, c, d, e, and f are each independently 0 or 1,
provided that at least one of a and b is 1; or a
physiologically acceptable salt thereof, and wherein
said peptide is capable of modulating NGF activity.

147.   The composition of matter of Claim 146 of
the formula: X^1-F^1 or F^1-X^2 or a physiologically
acceptable salt thereof.

148.   The composition of matter of Claim 146 of
the formula: $F^1-(L^1)_c-P^1$ or a physiologically
acceptable salt thereof.

149.   The composition of matter of Claim 146 of
the formula: $F^1-(L^1)_c-P^1-(L^2)_d-P^2$ or a physiologically
acceptable salt thereof.

150.   The composition of matter of Claim 146 of
the formula: $P^1-(L^1)_c-F^1-(L^2)_d-P^2$ or a physiologically
acceptable salt thereof.

139

151. The composition of matter of Claim 146, 147, 148, 149, or 150, wherein F^1 is an Fc domain or fragment thereof.

5

152. A polynucleotide encoding the peptide or polypeptide according to Claim 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, or 151.

10   153. An expression vector comprising the polynucleotide of Claim 39.

154. A host cell comprising the expression vector of Claim 153.

15

155. The host cell according to Claim 154 wherein the cell is a prokaryotic cell.

156. The host cell according to Claim 155 wherein
20 the cell is an *E. coli* cell.

157. The host cell according to Claim 154 wherein the cell is a eukaryotic cell.

FIG. 1A

FIG. 1B

N-terminus

peptide

linker

Fc

Fc

linker

peptide

C-terminus
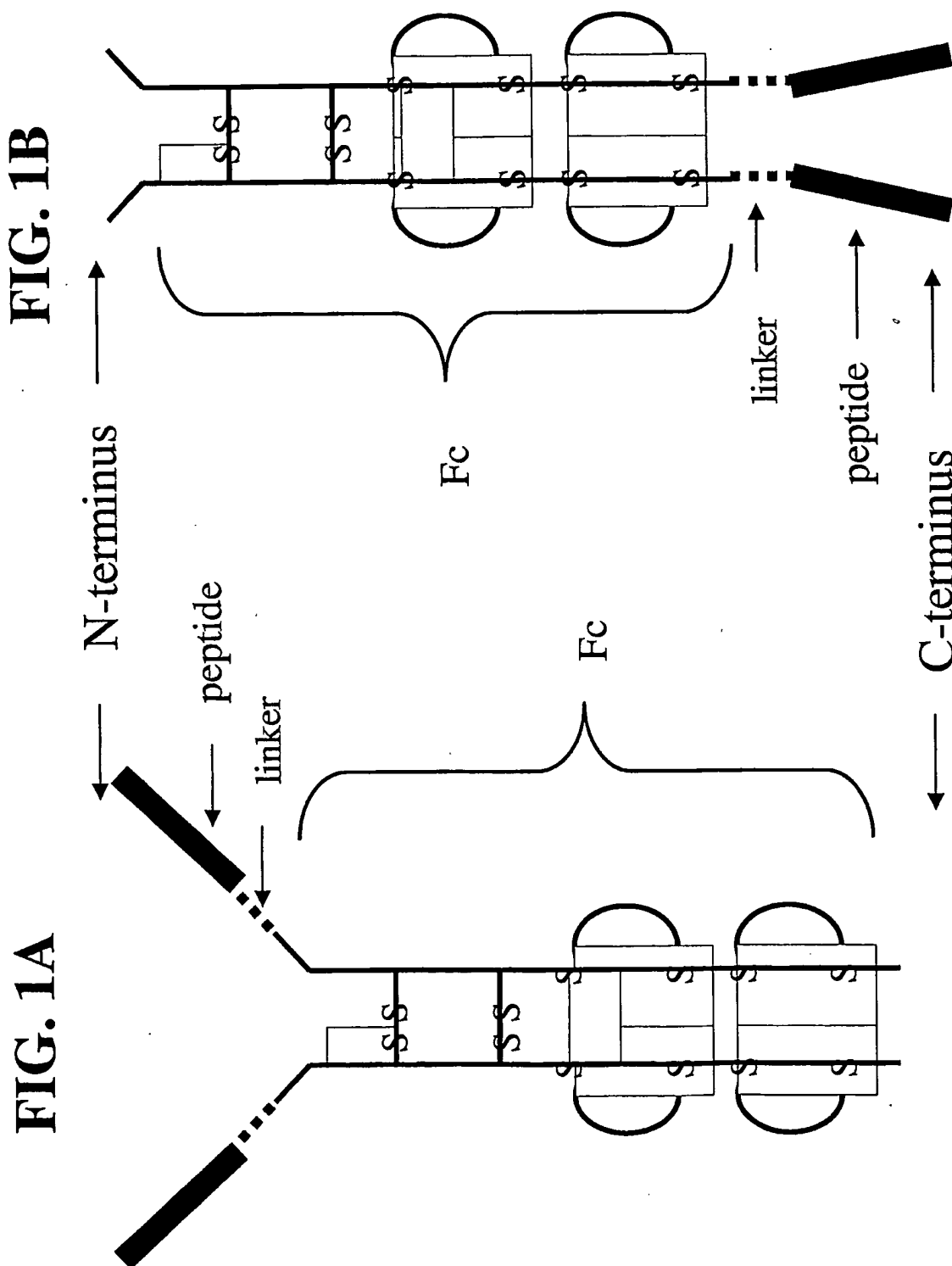
FIG. 2A

```
     ATGGACAAAACTCACACATGTCCACCTTGTCCAGCTCCGGAACTCCTGGGGGGACCGTCA
  1  ---------+---------+---------+---------+---------+---------+ 60
     TACCTGTTTTGAGTGTGTACAGGTGGAACAGGTCGAGGCCTTGAGGACCCCCCTGGCAGT

      M   D   K   T   H   T   C   P   P   C   P   A   P   E   L   L   G   G   P   S


     GTCTTCCTCTTCCCCCCAAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTC
 61  ---------+---------+---------+---------+---------+---------+ 120
     CAGAAGGAGAAGGGGGGTTTTTGGGTTCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCAG

      V   F   L   F   P   P   K   P   K   D   T   L   M   I   S   R   T   P   E   V


     ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTG
121  ---------+---------+---------+---------+---------+---------+ 180
     TGTACGCACCACCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCAC

      T   C   V   V   V   D   V   S   H   E   D   P   E   V   K   F   N   W   Y   V


     GACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
181  ---------+---------+---------+---------+---------+---------+ 240
     CTGCCGCACCTCCACGTATTACGGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTCGTGC

      D   G   V   E   V   H   N   A   K   T   K   P   R   E   E   Q   Y   N   S   T


     TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTAC
241  ---------+---------+---------+---------+---------+---------+ 300
     ATGGCACACCAGTCGCAGGAGTGGCAGGACGTGGTCCTGACCGACTTACCGTTCCTCATG

      Y   R   V   V   S   V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y


     AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCC
301  ---------+---------+---------+---------+---------+---------+ 360
     TTCACGTTCCAGAGGTTGTTTCGGGAGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTTCGG

      K   C   K   V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A


     AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACC
361  ---------+---------+---------+---------+---------+---------+ 420
     TTTCCCGTCGGGGCTCTTGGTGTCCACATGTGGGACGGGGGTAGGGCCCTACTCGACTGG

      K   G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L   T


     AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG
421  ---------+---------+---------+---------+---------+---------+ 480
     TTCTTGGTCCAGTCGGACTGGACGGACCAGTTTCCGAAGATAGGGTCGCTGTAGCGGCAC

      K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D   I   A   V
```

FIG. 2B

```
     GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGAC
481  ---------+---------+---------+---------+---------+---------+ 540
     CTCACCCTCTCGTTACCCGTCGGCCTCTTGTTGATGTTCTGGTGCGGAGGGCACGACCTG

     E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T   P   P   V   L   D


     TCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAG
541  ---------+---------+---------+---------+---------+---------+ 600
     AGGCTGCCGAGGAAGAAGGAGATGTCGTTCGAGTGGCACCTGTTCTCGTCCACCGTCGTC

     S   D   G   S   F   F   L   Y   S   K   L   T   V   D   K   S   R   W   Q   Q


     GGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
601  ---------+---------+---------+---------+---------+---------+ 660
     CCCTTGCAGAAGAGTACGAGGCACTACGTACTCCGAGACGTGTTGGTGATGTGCGTCTTC

     G   N   V   F   S   C   S   V   M   H   E   A   L   H   N   H   Y   T   Q   K


     AGCCTCTCCCTGTCTCCGGGTAAA
661  ---------+---------+----  684
     TCGGAGAGGGACAGAGGCCCATTT

     S   L   S   L   S   P   G   K
```

FIG. 3A

```
XbaI                                    NdeI ApaLI
  |                                       |    |
TCTAGATTTGTTTTAACTAATTAAAGGAGGAATAACATATGGGTGCACAGAAAGCGGCCG
1 ---------+---------+---------+---------+---------+---------+ 60
AGATCTAAACAAAATTGATTAATTTCCTCCTTATTGTATACCCACGTGTCTTTCGCCGGC


      XhoI
        |
CAAAAAAACTCGAGGGTGGAGGCGGTGGGGACAAAACTCACACATGTCCACCTTGCCCAG
61 ---------+---------+---------+---------+---------+---------+ 120
GTTTTTTTGAGCTCCCACCTCCGCCACCCCTGTTTTGAGTGTGTACAGGTGGAACGGGTC


CACCTGAACTCCTGGGGGGACCGTCAGTTTTCCTCTTCCCCCCAAAACCCAAGGACACCC
121 ---------+---------+---------+---------+---------+---------+ 180
GTGGACTTGAGGACCCCCCTGGCAGTCAAAAGGAGAAGGGGGGTTTTGGGTTCCTGTGGG


TCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACC
181 ---------+---------+---------+---------+---------+---------+ 240
AGTACTAGAGGGCCTGGGGACTCCAGTGTACGCACCACCACCTGCACTCGGTGCTTCTGG


CTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGC
241 ---------+---------+---------+---------+---------+---------+ 300
GACTCCAGTTCAAGTTGACCATGCACCTGCCGCACCTCCACGTATTACGGTTCTGTTTCG


CGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACC
301 ---------+---------+---------+---------+---------+---------+ 360
GCGCCCTCCTCGTCATGTTGTCGTGCATGGCACACCAGTCGCAGGAGTGGCAGGACGTGG


AGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC
361 ---------+---------+---------+---------+---------+---------+ 420
TCCTGACCGACTTACCGTTCCTCATGTTCACGTTCCAGAGGTTGTTTCGGGAGGGTCGGG


CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCC
421 ---------+---------+---------+---------+---------+---------+ 480
GGTAGCTCTTTTGGTAGAGGTTTCGGTTTCCCGTCGGGGCTCTTGGTGTCCACATGTGGG


TGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG
481 ---------+---------+---------+---------+---------+---------+ 540
ACGGGGGTAGGGCCCTACTCGACTGGTTCTTGGTCCAGTCGGACTGGACGGACCAGTTTC
```

FIG. 3B

```
     GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACT
541  ---------+---------+---------+---------+---------+---------+ 600
     CGAAGATAGGGTCGCTGTAGCGGCACCTCACCCTCTCGTTACCCGTCGGCCTCTTGTTGA


     ACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA
601  ---------+---------+---------+---------+---------+---------+ 660
     TGTTCTGGTGCGGAGGGCACGACCTGAGGCTGCCGAGGAAGAAGGAGATGTCGTTCGAGT


     CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGG
661  ---------+---------+---------+---------+---------+---------+ 720
     GGCACCTGTTCTCGTCCACCGTCGTCCCCTTGCAGAAGAGTACGAGGCACTACGTACTCC


                                                         BamHI
                                                           |
     CTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATAATGGATCC
721  ---------+---------+---------+---------+---------+---------+ 780
     GAGACGTGTTGGTGATGTGCGTCTTCTCGGAGAGGGACAGAGGCCCATTTATTACCTAGG
```

FIG. 4A

```
XbaI                                  NdeI
  |                                     |
  TCTAGATTTGTTTTAACTAATTAAAGGAGGAATAACATATGGACAAAACTCACACATGTC
1 ---------+---------+---------+---------+---------+---------+ 60
  AGATCTAAACAAAATTGATTAATTTCCTCCTTATTGTATACCTGTTTTGAGTGTGTACAG


   CACCTTGTCCAGCTCCGGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCCAAAAC
61 ---------+---------+---------+---------+---------+---------+ 120
   GTGGAACAGGTCGAGGCCTTGAGGACCCCCCTGGCAGTCAGAAGGAGAAGGGGGGTTTTG


    CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGA
121 ---------+---------+---------+---------+---------+---------+ 180
    GGTTCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCAGTGTACGCACCACCACCTGCACT


    GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
181 ---------+---------+---------+---------+---------+---------+ 240
    CGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCTGCCGCACCTCCACGTATTAC


    CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCA
241 ---------+---------+---------+---------+---------+---------+ 300
    GGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTCGTGCATGGCACACCAGTCGCAGGAGT


    CCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAG
301 ---------+---------+---------+---------+---------+---------+ 360
    GGCAGGACGTGGTCCTGACCGACTTACCGTTCCTCATGTTCACGTTCCAGAGGTTGTTTC


    CCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCAC
361 ---------+---------+---------+---------+---------+---------+ 420
    GGGAGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTTCGGTTTCCCGTCGGGGCTCTTGGTG


    AGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCT
421 ---------+---------+---------+---------+---------+---------+ 480
    TCCACATGTGGGACGGGGGTAGGGCCCTACTCGACTGGTTCTTGGTCCAGTCGGACTGGA


    GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGC
481 ---------+---------+---------+---------+---------+---------+ 540
    CGGACCAGTTTCCGAAGATAGGGTCGCTGTAGCGGCACCTCACCCTCTCGTTACCCGTCG


    CGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCT
541 ---------+---------+---------+---------+---------+---------+ 600
    GCCTCTTGTTGATGTTCTGGTGCGGAGGGCACGACCTGAGGCTGCCGAGGAAGAAGGAGA
```

FIG. 4B

```
    ACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCG
601 ---------+---------+---------+---------+---------+---------+ 660
    TGTCGTTCGAGTGGCACCTGTTCTCGTCCACCGTCGTCCCCTTGCAGAAGAGTACGAGGC


    TGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTA
661 ---------+---------+---------+---------+---------+---------+ 720
    ACTACGTACTCCGAGACGTGTTGGTGATGTGCGTCTTCTCGGAGAGGGACAGAGGCCCAT


                   ApaLI                         XhoI     BamHI
                     |                             |        |
    AAGGTGGAGGTGGTGGTGCACAGAAAGCGGCCGCAAAAAAACTCGAGTAATGGATCC
721 ---------+---------+---------+---------+---------+------- 777
    TTCCACCTCCACCACCACGTGTCTTTCGCCGGCGTTTTTTTGAGCTCATTACCTAGG
```

FIG. 5
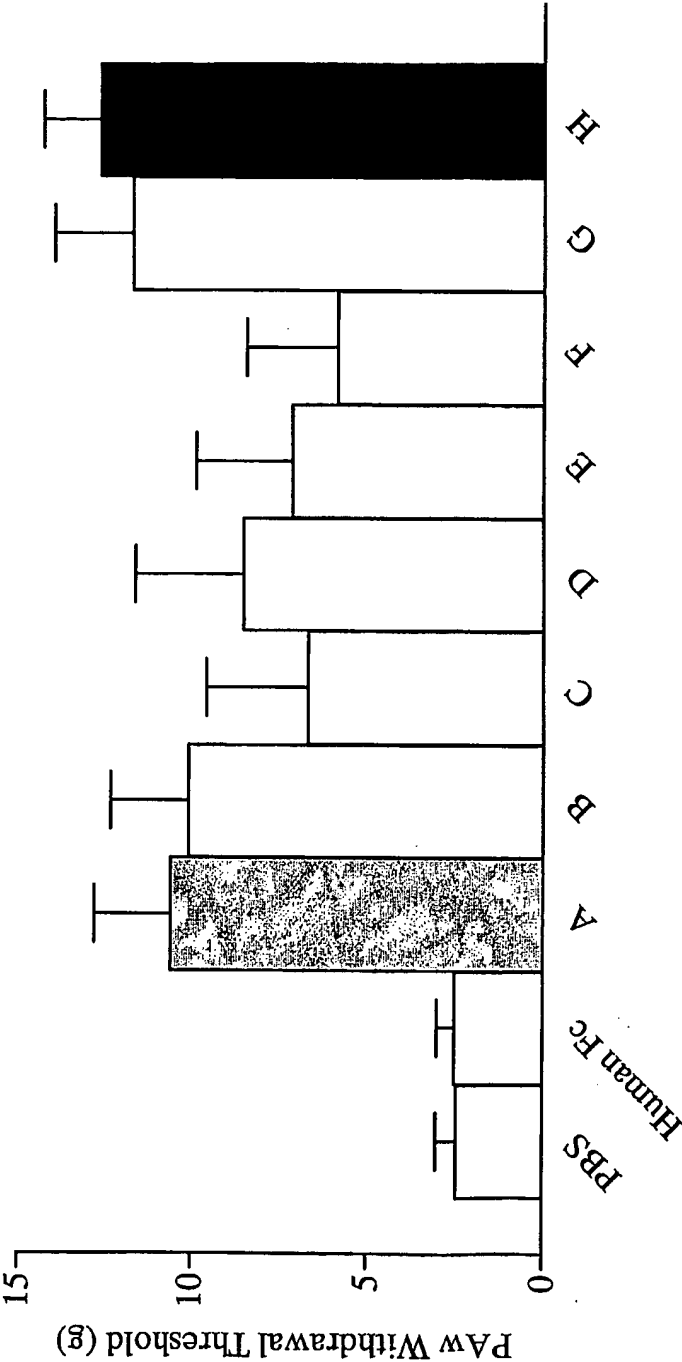
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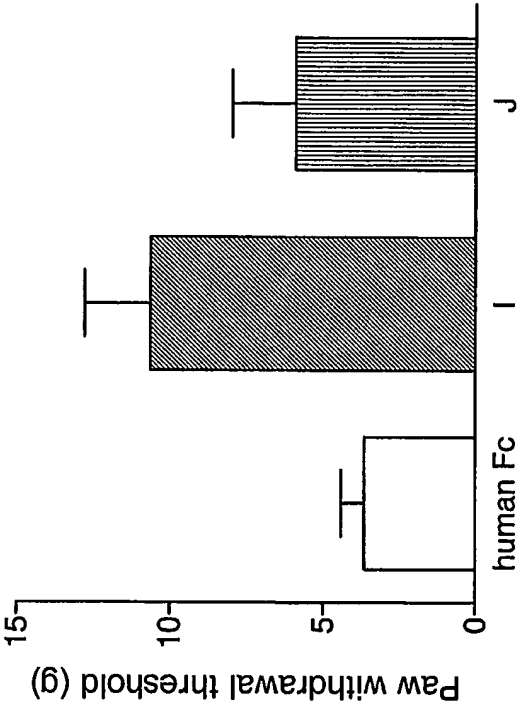
SEQUENCE LISTING

<110> Amgen Inc.

<120> Peptides and Related Molecules That Modulate Nerve Growth Factor
Activity

<130> A-827PCT

<140> Not Yet Assigned

<141> 2003-09-19

<150> Not Yet Assigned

<151> 2003-09-18

<150> 60/412,524

<151> 2002-09-19

<160> 286

<170> PatentIn version 3.1

<210> 1

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occuring sequence

<400> 1

Thr Gly Tyr Thr Glu Tyr Thr Glu Glu Trp Pro Met Gly Phe Gly Tyr
1   5   10   15

```
Gln Trp Ser Phe
            20


<210>   2

<211>   20

<212>   PRT

<213>   Artificial Sequence



<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   2

Thr Asp Trp Leu Ser Asp Phe Pro Phe Tyr Glu Gln Tyr Phe Gly Leu
1               5                   10                  15


Met Pro Pro Gly
            20


<210>   3

<211>   20

<212>   PRT

<213>   Artificial Sequence



<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   3

Phe Met Arg Phe Pro Asn Pro Trp Lys Leu Val Glu Pro Pro Gln Gly
1               5                   10                  15


Trp Tyr Tyr Gly
            20


<210>   4

<211>   20

<212>   PRT

<213>   Artificial Sequence
```

<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   4

Val Val Lys Ala Pro His Phe Glu Phe Leu Ala Pro Pro His Phe His
1   5   10   15

Glu Phe Pro Phe
            20

<210>   5

<211>   20

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   5

Phe Ser Tyr Ile Trp Ile Asp Glu Thr Pro Ser Asn Ile Asp Arg Tyr
1   5   10   15

Met Leu Trp Leu
            20

<210>   6

<211>   20

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   6

Val Asn Phe Pro Lys Val Pro Glu Asp Val Glu Pro Trp Pro Trp Ser
1   5   10   15

Leu Lys Leu Tyr
            20

<210> 7

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 7

Thr Trp His Pro Lys Thr Tyr Glu Glu Phe Ala Leu Pro Phe Phe Val
1   5   10   15

Pro Glu Ala Pro
            20

<210> 8

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 8

Trp His Phe Gly Thr Pro Tyr Ile Gln Gln Gln Pro Gly Val Tyr Trp
1   5   10   15

Leu Gln Ala Pro
            20

<210> 9

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural

ly occurring sequence

<400> 9

Val Trp Asn Tyr Gly Pro Phe Phe Met Asn Phe Pro Asp Ser Thr Tyr
1   5   10   15

Phe Leu His Glu
            20

<210> 10

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 10

Trp Arg Ile His Ser Lys Pro Leu Asp Tyr Ser His Val Trp Phe Phe
1   5   10   15

Pro Ala Asp Phe
            20

<210> 11

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 11

Phe Trp Asp Gly Asn Gln Pro Pro Asp Ile Leu Val Asp Trp Pro Trp
1   5   10   15

Asn Pro Pro Val
            20

<210> 12

```
<211>   20

<212>   PRT

<213>   Artificial Sequence


<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   12

Phe Tyr Ser Leu Glu Trp Leu Lys Asp His Ser Glu Phe Phe Gln Thr
1               5                   10                  15


Val Thr Glu Trp
            20


<210>   13

<211>   20

<212>   PRT

<213>   Artificial Sequence


<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   13

Gln Phe Met Glu Leu Leu Lys Phe Phe Asn Ser Pro Gly Asp Ser Ser
1               5                   10                  15


His His Phe Leu
            20


<210>   14

<211>   20

<212>   PRT

<213>   Artificial Sequence


<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   14
```

```
Thr Asn Val Asp Trp Ile Ser Asn Asn Trp Glu His Met Lys Ser Phe
1               5                   10              15

Phe Thr Glu Asp
            20
```

<210> 15

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 15

```
Pro Asn Glu Lys Pro Tyr Gln Met Gln Ser Trp Phe Pro Pro Asp Trp
1               5                   10              15

Pro Val Pro Tyr
            20
```

<210> 16

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 16

```
Trp Ser His Thr Glu Trp Val Pro Gln Val Trp Trp Lys Pro Pro Asn
1               5                   10              15

His Phe Tyr Val
            20
```

<210> 17

<211> 20

<210> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 17

Trp Gly Glu Trp Ile Asn Asp Ala Gln Val His Met His Glu Gly Phe
1   5   10   15

Ile Ser Glu Ser
            20

<210> 18

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 18

Val Pro Trp Glu His Asp His Asp Leu Trp Glu Ile Ile Ser Gln Asp
1   5   10   15

Trp His Ile Ala
            20

<210> 19

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 19

Val Leu His Leu Gln Asp Pro Arg Gly Trp Ser Asn Phe Pro Pro Gly

1   5   10   15

Val Leu Glu Leu
            20

<210> 20

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 20

Ile His Gly Cys Trp Phe Thr Glu Glu Gly Cys Val Trp Gln
1   5   10

<210> /21

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 21

Tyr Met Gln Cys Gln Phe Ala Arg Asp Gly Cys Pro Gln Trp
1   5   10

<210> 22

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 22

Lys Leu Gln Cys Gln Tyr Ser Glu Ser Gly Cys Pro Thr Ile
1   5   10

<210> 23

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 23

Phe Leu Gln Cys Glu Ile Ser Gly Gly Ala Cys Pro Ala Pro
1   5   10

<210> 24

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 24

Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro Asp Leu
1   5   10

<210> 25

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 25

```
Lys Leu Gln Cys Glu Phe Ser Thr Gln Gly Cys Pro Asp Leu
1               5                   10
```

<210> 26

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 26

```
Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro Trp Leu
1               5                   10
```

<210> 27

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 27

```
Ile Gln Gly Cys Trp Phe Thr Glu Glu Gly Cys Pro Trp Gln
1               5                   10
```

<210> 28

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 28

```
Ser Phe Asp Cys Asp Asn Pro Trp Gly His Val Leu Gln Ser Cys Phe
1               5               10              15

Gly Phe
```

<210> 29

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 29

```
Ser Phe Asp Cys Asp Asn Pro Trp Gly His Lys Leu Gln Ser Cys Phe
1               5               10              15

Gly Phe
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<210> 30

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 30

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Met Thr Gly Tyr Thr Glu Tyr Thr Glu Glu Trp Pro Met Gly Phe Gly
1               5               10              15

Tyr Gln Trp Ser Phe
            20
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<210> 31

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 31

Met Thr Asp Trp Leu Ser Asp Phe Pro Phe Tyr Glu Gln Tyr Phe Gly
1   5   10   15

Leu Met Pro Pro Gly
            20

<210> 32

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 32

Met Phe Met Arg Phe Pro Asn Pro Trp Lys Leu Val Glu Pro Pro Gln
1   5   10   15

Gly Trp Tyr Tyr Gly
            20

<210> 33

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 33

Met Val Val Lys Ala Pro His Phe Glu Phe Leu Ala Pro Pro His Phe
1   5   10   15

His Glu Phe Pro Phe
            20

<210>   34

<211>   21

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   34

Met Phe Ser Tyr Ile Trp Ile Asp Glu Thr Pro Ser Asn Ile Asp Arg
1   5   10   15

Tyr Met Leu Trp Leu
            20

<210>   35

<211>   21

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   35

Met Val Asn Phe Pro Lys Val Pro Glu Asp Val Glu Pro Trp Pro Trp
1   5   10   15

Ser Leu Lys Leu Tyr
            20

<210>   36

<211>   21

<212>   PRT

<213>   Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 36

Met Thr Trp His Pro Lys Thr Tyr Glu Glu Phe Ala Leu Pro Phe Phe
1   5   10   15

Val Pro Glu Ala Pro
            20

<210> 37

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 37

Met Trp His Phe Gly Thr Pro Tyr Ile Gln Gln Gln Pro Gly Val Tyr
1   5   10   15

Trp Leu Gln Ala Pro
            20

<210> 38

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 38

Met Val Trp Asn Tyr Gly Pro Phe Phe Met Asn Phe Pro Asp Ser Thr
1   5   10   15

Tyr Phe Leu His Glu

20

<210> 39

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 39

Met Trp Arg Ile His Ser Lys Pro Leu Asp Tyr Ser His Val Trp Phe
1   5   10   15

Phe Pro Ala Asp Phe
            20

<210> 40

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 40

Met Phe Trp Asp Gly Asn Gln Pro Pro Asp Ile Leu Val Asp Trp Pro
1   5   10   15

Trp Asn Pro Pro Val
            20

<210> 41

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 41

Met Phe Tyr Ser Leu Glu Trp Leu Lys Asp His Ser Glu Phe Phe Gln
1   5   10   15

Thr Val Thr Glu Trp
            20

<210> 42

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 42

Met Gln Phe Met Glu Leu Leu Lys Phe Phe Asn Ser Pro Gly Asp Ser
1   5   10   15

Ser His His Phe Leu
            20

<210> 43

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 43

Met Thr Asn Val Asp Trp Ile Ser Asn Asn Trp Glu His Met Lys Ser
1   5   10   15

Phe Phe Thr Glu Asp
            20

<210> 44

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 44

Met Pro Asn Glu Lys Pro Tyr Gln Met Gln Ser Trp Phe Pro Pro Asp
1   5   10   15

Trp Pro Val Pro Tyr
            20

<210> 45

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 45

Met Trp Ser His Thr Glu Trp Val Pro Gln Val Trp Trp Lys Pro Pro
1   5   10   15

Asn His Phe Tyr Val
            20

<210> 46

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural

ly occurring sequence

<400> 46

Met Trp Gly Glu Trp Ile Asn Asp Ala Gln Val His Met His Glu Gly
1   5   10   15

Phe Ile Ser Glu Ser
           20

<210> 47

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 47

Met Val Pro Trp Glu His Asp His Asp Leu Trp Glu Ile Ile Ser Gln
1   5   10   15

Asp Trp His Ile Ala
           20

<210> 48

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 48

Met Val Leu His Leu Gln Asp Pro Arg Gly Trp Ser Asn Phe Pro Pro
1   5   10   15

Gly Val Leu Glu Leu
           20

<210> 49

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 49

Met Ile His Gly Cys Trp Phe Thr Glu Glu Gly Cys Val Trp Gln
1   5   10   15

<210> 50

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 50

Met Tyr Met Gln Cys Gln Phe Ala Arg Asp Gly Cys Pro Gln Trp
1   5   10   15

<210> 51

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 51

Met Lys Leu Gln Cys Gln Tyr Ser Glu Ser Gly Cys Pro Thr Ile
1   5   10   15

<210> 52

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 52

Met Phe Leu Gln Cys Glu Ile Ser Gly Gly Ala Cys Pro Ala Pro
1   5   10   15

<210> 53

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 53

Met Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro Asp Leu
1   5   10   15

<210> 54

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 54

Met Lys Leu Gln Cys Glu Phe Ser Thr Gln Gly Cys Pro Asp Leu
1   5   10   15

<210> 55

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 55

Met Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro Trp Leu
1   5   10   15

<210> 56

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 56

Met Ile Gln Gly Cys Trp Phe Thr Glu Glu Gly Cys Pro Trp Gln
1   5   10   15

<210> 57

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 57

Met Ser Phe Asp Cys Asp Asn Pro Trp Gly His Val Leu Gln Ser Cys
1   5   10   15

Phe Gly Phe

<210> 58

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 58

Met Ser Phe Asp Cys Asp Asn Pro Trp Gly His Lys Leu Gln Ser Cys
1   5   10   15

Phe Gly Phe

<210> 59

<211> 684

<212> DNA

<213> Homo sapiens

<400> 59
atggacaaaa ctcacacatg tccaccttgt ccagctccgg aactcctggg gggaccgtca   60

gtcttcctct tccccccaaa acccaaggac accctcatga tctcccggac ccctgaggtc   120

acatgcgtgg tggtggacgt gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg   180

gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg   240

taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtac   300

aagtgcaagg tctccaacaa agccctccca gcccccatcg agaaaaccat ctccaaagcc   360

aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga tgagctgacc   420

aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg   480

gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac   540

tccgacggct ccttcttcct ctacagcaag ctcaccgtgg acaagagcag gtggcagcag   600

gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag   660

agcctctccc tgtctccggg taaa   684

<210> 60

<211> 228

<212> PRT

<213> Homo sapiens

<400> 60

Met	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu
1				5					10					15	

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20   25   30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35   40   45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50   55   60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65   70   75   80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
            85   90   95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100   105   110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            115   120   125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130   135   140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145   150   155   160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
            165   170   175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180   185   190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            195   200   205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            210   215   220

Ser Pro Gly Lys
225

<210>   61

<211>   779

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Expression vector component

<400>   61
tctagatttg ttttaactaa ttaaaggagg aataacatat gggtgcacag aaagcggccg   60

caaaaaaact cgagggtgga ggcggtgggg acaaaactca cacatgtcca ccttgcccag   120

cacctgaact cctggggggga ccgtcagttt tcctcttccc cccaaaaccc aaggacaccc   180

tcatgatctc ccggaccect gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc   240

ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc aagacaaagc   300

cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc gtcctgcaca   360

ggactggctg aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc   420

catcgagaaa accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct   480

gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg   540

cttctatccc agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta   600

caagaccacg cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac   660

cgtggacaag agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc   720

tctgcacaac cactacacgc agaagagcct ctccctgtct ccgggtaaat aatggatcc   779

<210>   62

<211>   780

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Expression vector component

<400>   62
agatctaaac aaaattgatt aatttcctcc ttattgtata cccacgtgtc tttcgccggc   60

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gtttttttga gctcccacct ccgccacccc tgttttgagt gtgtacaggt ggaacgggtc    120

gtggacttga ggacccccct ggcagtcaaa aggagaaggg gggtttttggg ttcctgtggg    180

agtactagag ggcctgggga ctccagtgta cgcaccacca cctgcactcg gtgcttctgg    240

gactccagtt caagttgacc atgcacctgc cgcacctcca cgtattacgg ttctgtttcg    300

gcgccctcct cgtcatgttg tcgtgcatgg cacaccagtc gcaggagtgg caggacgtgg    360

tcctgaccga cttaccgttc ctcatgttca cgttccagag gttgtttcgg gagggtcggg    420

ggtagctctt ttggtagagg tttcggtttc ccgtcggggc tcttggtgtc cacatgtggg    480

acgggggtag ggccctactc gactggttct tggtccagtc ggactggacg gaccagtttc    540

cgaagatagg gtcgctgtag cggcacctca ccctctcgtt acccgtcggc ctcttgttga    600

tgttctggtg cggagggcac gacctgaggc tgccgaggaa gaaggagatg tcgttcgagt    660

ggcacctgtt ctcgtccacc gtcgtcccct tgcagaagag tacgaggcac tacgtactcc    720

gagacgtgtt ggtgatgtgc gtcttctcgg agagggacag aggcccattt attacctagg    780
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<210>   63

<211>   57

<212>   DNA

<213>   Artificial Sequence


<220>

<223>   Encodes for therapeutically active peptides with methionine resid
        ue at N-terminus

<400>   63
atgattcatg gttgttggtt tacagaagaa ggttgtgttt ggcaactcga gggtgga         57


<210>   64

<211>   57

<212>   DNA

<213>   Artificial Sequence


<220>

<223>   Encodes for therapeutically active peptides with methionine resid
        ue at N-terminus

<400>   64
atgtatatgc aatgtcaatt tgctcgtgat ggttgtccac aatggctcga gggtgga         57
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<210> 65

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine residue at N-terminus

<400> 65
atgaaattac aatgtcaata ttctgaatct ggttgtccaa caattctcga gggtgga   57

<210> 66

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine residue at N-terminus

<400> 66
atgtttttac aatgtgaaat ttctggtggt gcttgtccag ctccactcga gggtgga   57

<210> 67

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine residue at N-terminus

<400> 67
atgaaattac aatgtgaatt ttctacttct ggttgtccag atttactcga gggtgga   57

<210> 68

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
      ue at N-terminus

<400> 68
atgaaattac aatgtgaatt ttctactcaa ggttgtccag atttactcga gggtgga   57

<210> 69

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
      ue at N-terminus

<400> 69
atgaaattac aatgtgaatt ttctacttct ggttgtcctt ggttactcga gggtgga   57

<210> 70

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
      ue at N-terminus

<400> 70
atgattcaag gttgttggtt tactgaagaa ggttgtcctt ggcaactcga gggtgga   57

<210> 71

<211> 69

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid

       ue at N-terminus

<400> 71
atgtcttttg attgtgataa tccttggggt catgttttac aatcttgttt tggttttctc   60

gagggtgga   69

<210> 72

<211> 69

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400> 72
atgtcttttg attgtgataa tccttggggt cataaattac aatcttgttt tggttttctc   60

gagggtgga   69

<210> 73

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400> 73
atgacaggtt atacagaata tacagaagaa tggccaatgg gttttggtta tcaatggtcc   60

tttctcgagg gtgga   75

<210> 74

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400>   74
atgacagatt ggttatctga ttttccattc tatgaacaat actttggttt aatgccacct   60

ggtctcgagg gtgga   75

<210>   75

<211>   75

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Encodes for therapeutically active peptides with methionine resid
        ue at N-terminus

<400>   75
atgtttatgc gttttcctaa cccatggaaa ttagttgaac cacctcaagg ttggtactat   60

ggtctcgagg gtgga   75

<210>   76

<211>   75

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Encodes for therapeutically active peptides with methionine resid
        ue at N-terminus

<400>   76
atggttgtta aagctccaca ttttgaattc ttagctccac ctcattttca tgaatttcca   60

tttctcgagg gtgga   75

<210>   77

<211>   75

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Encodes for therapeutically active peptides with methionine resid
        ue at N-terminus

<400> 77
atgttttctt atatttggat tgatgaaact ccgtctaaca ttgatcgtta tatgctgtgg   60

ctgctcgagg gtgga   .   75

<210> 78   .

<211> 74

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400> 78
tggttaactt tccgaaagtt ccggaagatg ttgaaccgtg gccgtggtct ctgaaactgt   60

atctcgaggg tgga   74

<210> 79

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400> 79
atgacttggc acccgaaaac ttatgaagaa tttgctctgc cgtttttttgt tccggaagct   60

ccgctcgagg gtgga   75

<210> 80

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400> 80

atgtggcatt ttggtactcc atatattcaa caacaaccag gtgtttattg gttacaagct   60

ccactcgagg gtgga   75

<210>   81

<211>   75

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Encodes for therapeutically active peptides with methionine resid
         ue at N-terminus

<400>   81
atggtttgga attatggtcc atttttttatg aattttccag attctactta tttttttacat   60

gaactcgagg gtgga   75

<210>   82

<211>   75

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Encodes for therapeutically active peptides with methionine resid
         ue at N-terminus

<400>   82
atgtggcgta ttcattctaa accattagat tattctcatg tttggttttt tccagctgat   60

tttctcgagg gtgga   75

<210>   83

<211>   75

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Encodes for therapeutically active peptides with methionine resid
         ue at N-terminus

<400>   83
atgtttttggg atggtaatca accaccagat attttagttg attggccatg gaatccacca   60

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gttctcgagg gtgga                                                         75


<210>  84

<211>  75

<212>  DNA

<213>  Artificial Sequence


<220>

<223>  Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400>  84
atgttttatt ctttagaatg gttaaaagat cattctgaat tttttcaaac tgttactgaa        60

tggctcgagg gtgga                                                         75


<210>  85

<211>  75

<212>  DNA

<213>  Artificial Sequence


<220>

<223>  Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400>  85
atgcaattta tggaattact gaaattcttt aattctccag gtgattcttc tcatcacttc        60

ttactcgagg gtgga                                                         75


<210>  86

<211>  75

<212>  DNA

<213>  Artificial Sequence


<220>

<223>  Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400>  86
atgactaatg ttgattggat ttctaataat tgggaacata tgaaatcttt ttttactgaa        60
```

```
gatctcgagg gtgga                                                    75
```

<210> 87

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

```
<400>  87
atgccaaatg aaaaaccata tcaaatgcaa tcttggtttc caccagattg gccagttcca    60

tatctcgagg gtgga                                                    75
```

<210> 88

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

```
<400>  88
atgtggtctc atactgaatg ggttccacaa gtttggtgga aaccaccaaa tcatttttat    60

gttctcgagg gtgga                                                    75
```

<210> 89

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

```
<400>  89
atgtggggtg aatggattaa tgatgctcaa gttcacatgc atgaaggttt tatttctgaa    60

tctctcgagg gtgga                                                    75
```

```
<210>  90

<211>  75

<212>  DNA

<213>  Artificial Sequence


<220>

<223>  Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400>  90
atggttccat gggaacatga tcatgattta tgggaaatta tttctcaaga ttggcatatt        60

gctctcgagg gtgga                                                         75


<210>  91

<211>  75

<212>  DNA

<213>  Artificial Sequence


<220>

<223>  Encodes for therapeutically active peptides with methionine resid
       ue at N-terminus

<400>  91
atggttttac atttacaaga tccacgtggt tggtctaatt ttccaccagg tgttttagaa        60

ttactcgagg gtgga                                                         75


<210>  92

<211>  19

<212>  PRT

<213>  Artificial Sequence


<220>

<223>  Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400>  92

Met Ile His Gly Cys Trp Phe Thr Glu Glu Gly Cys Val Trp Gln Leu
1               5                   10                  15
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Glu Gly Gly

<210> 93

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 93

Met Tyr Met Gln Cys Gln Phe Ala Arg Asp Gly Cys Pro Gln Trp Leu
1   5   10   15

Glu Gly Gly

<210> 94

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 94

Met Lys Leu Gln Cys Gln Tyr Ser Glu Ser Gly Cys Pro Thr Ile Leu
1   5   10   15

Glu Gly Gly

<210> 95

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 95

Met Phe Leu Gln Cys Glu Ile Ser Gly Gly Ala Cys Pro Ala Pro Leu
1   5   10   15

Glu Gly Gly

<210> 96

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 96

Met Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro Asp Leu Leu
1   5   10   15

Glu Gly Gly

<210> 97

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 97

Met Lys Leu Gln Cys Glu Phe Ser Thr Gln Gly Cys Pro Asp Leu Leu
1   5   10   15

Glu Gly Gly

<210> 98

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 98

Met Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro Trp Leu Leu
1   5   10   15

Glu Gly Gly

<210> 99

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 99

Met Ile Gln Gly Cys Trp Phe Thr Glu Glu Gly Cys Pro Trp Gln Leu
1   5   10   15

Glu Gly Gly

<210> 100

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   100

Met Ser Phe Asp Cys Asp Asn Pro Trp Gly His Val Leu Gln Ser Cys
1   5   10   15

Phe Gly Phe Leu Glu Gly Gly
            20

<210>   101

<211>   23

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   101

Met Ser Phe Asp Cys Asp Asn Pro Trp Gly His Lys Leu Gln Ser Cys
1   5   10   15

Phe Gly Phe Leu Glu Gly Gly
            20

<210>   102

<211>   25

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natural
        ly occurring sequence

<400>   102

Met Thr Gly Tyr Thr Glu Tyr Thr Glu Glu Trp Pro Met Gly Phe Gly
1   5   10   15

Tyr Gln Trp Ser Phe Leu Glu Gly Gly
            20   25

<210> 103

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 103

Met Thr Asp Trp Leu Ser Asp Phe Pro Phe Tyr Glu Gln Tyr Phe Gly
1   5   10   15

Leu Met Pro Pro Gly Leu Glu Gly Gly Gly
            20   25

<210> 104

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 104

Met Phe Met Arg Phe Pro Asn Pro Trp Lys Leu Val Glu Pro Pro Gln
1   5   10   15

Gly Trp Tyr Tyr Gly Leu Glu Gly Gly
            20   25

<210> 105

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural

ly occurring sequence

<400> 105

Met Val Val Lys Ala Pro His Phe Glu Phe Leu Ala Pro Pro His Phe
1   5   10   15

His Glu Phe Pro Phe Leu Glu Gly Gly
              20   25

<210> 106

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 106

Met Phe Ser Tyr Ile Trp Ile Asp Glu Thr Pro Ser Asn Ile Asp Arg
1   5   10   15

Tyr Met Leu Trp Leu Leu Glu Gly Gly
              20   25

<210> 107

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 107

Met Val Asn Phe Pro Lys Val Pro Glu Asp Val Glu Pro Trp Pro Trp
1   5   10   15

Ser Leu Lys Leu Tyr Leu Glu Gly Gly Gly
              20   25

<210> 108

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 108

Met Thr Trp His Pro Lys Thr Tyr Glu Glu Phe Ala Leu Pro Phe Phe
1   5   10   15

Val Pro Glu Ala Pro Leu Glu Gly Gly
            20   25

<210> 109

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 109

Met Trp His Phe Gly Thr Pro Tyr Ile Gln Gln Gln Pro Gly Val Tyr
1   5   10   15

Trp Leu Gln Ala Pro Leu Glu Gly Gly
            20   25

<210> 110

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 110

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Met Val Trp Asn Tyr Gly Pro Phe Phe Met Asn Phe Pro Asp Ser Thr
1               5                   10                  15

Tyr Phe Leu His Glu Leu Glu Gly Gly
            20                  25
```

<210> 111

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 111

```
Met Trp Arg Ile His Ser Lys Pro Leu Asp Tyr Ser His Val Trp Phe
1               5                   10                  15

Phe Pro Ala Asp Phe Leu Glu Gly Gly
            20                  25
```

<210> 112

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 112

```
Met Phe Trp Asp Gly Asn Gln Pro Pro Asp Ile Leu Val Asp Trp Pro
1               5                   10                  15

Trp Asn Pro Pro Val Leu Glu Gly Gly
            20                  25
```

<210> 113

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 113

Met Phe Tyr Ser Leu Glu Trp Leu Lys Asp His Ser Glu Phe Phe Gln
1   5   10   15

Thr Val Thr Glu Trp Leu Glu Gly Gly
            20   25

<210> 114

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 114

Met Gln Phe Met Glu Leu Leu Lys Phe Phe Asn Ser Pro Gly Asp Ser
1   5   10   15

Ser His His Phe Leu Leu Glu Gly Gly
            20   25

<210> 115

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 115

His Met Thr Asn Val Asp Trp Ile Ser Asn Asn Trp Glu His Met Lys

1   5   10   15

Ser Phe Phe Thr Glu Asp Leu Glu Gly Gly
            20   25

<210> 116

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 116

Met Pro Asn Glu Lys Pro Tyr Gln Met Gln Ser Trp Phe Pro Pro Asp
1   5   10   15

Trp Pro Val Pro Tyr Leu Glu Gly Gly
            20   25

<210> 117

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
       ly occurring sequence

<400> 117

Met Trp Ser His Thr Glu Trp Val Pro Gln Val Trp Trp Lys Pro Pro
1   5   10   15

Asn His Phe Tyr Val Leu Glu Gly Gly
            20   25

<210> 118

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 118

Met Trp Gly Glu Trp Ile Asn Asp Ala Gln Val His Met His Glu Gly
1   5   10   15

Phe Ile Ser Glu Ser Leu Glu Gly Gly
            20   25

<210> 119

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 119

Met Val Pro Trp Glu His Asp His Asp Leu Trp Glu Ile Ile Ser Gln
1   5   10   15

Asp Trp His Ile Ala Leu Glu Gly Gly
            20   25

<210> 120

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
      ly occurring sequence

<400> 120

Met Val Leu His Leu Gln Asp Pro Arg Gly Trp Ser Asn Phe Pro Pro
1   5   10   15

Gly Val Leu Glu Leu Leu Glu Gly Gly
            20   25

<210>   121

<211>   777

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Expression vector

<400>   121
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caccttgtcc agctccggaa ctcctggggg gaccgtcagt cttcctcttc cccccaaaac   120

ccaaggacac cctcatgatc tcccggaccc ctgaggtcac atgcgtggtg gtggacgtga   180

gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag gtgcataatg   240

ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc agcgtcctca   300

ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc tccaacaaag   360

ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc cgagaaccac   420

aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc agcctgacct   480

gcctggtcaa aggcttctat cccagcgaca tcgccgtgga gtgggagagc aatgggcagc   540

cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc ttcttcctct   600

acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc tcatgctccg   660

tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg tctccgggta   720

aaggtggagg tggtggtgca cagaaagcgg ccgcaaaaaa actcgagtaa tggatcc   777

<210>   122

<211>   777

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Expression vector

<400>   122
agatctaaac aaaattgatt aatttcctcc ttattgtata cctgttttga gtgtgtacag   60

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gtggaacagg tcgaggcctt gaggacccc ctggcagtca gaaggagaag gggggtttg    120

ggttcctgtg ggagtactag agggcctggg gactccagtg tacgcaccac cacctgcact   180

cggtgcttct gggactccag ttcaagttga ccatgcacct gccgcacctc cacgtattac   240

ggttctgttt cggcgccctc ctcgtcatgt tgtcgtgcat ggcacaccag tcgcaggagt   300

ggcaggacgt ggtcctgacc gacttaccgt tcctcatgtt cacgttccag aggttgtttc   360

gggagggtcg ggggtagctc ttttggtaga ggtttcggtt tcccgtcggg gctcttggtg   420

tccacatgtg ggacgggggt agggccctac tcgactggtt cttggtccag tcggactgga   480

cggaccagtt ccgaagata gggtcgctgt agcggcacct caccctctcg ttacccgtcg   540

gcctcttgtt gatgttctgg tgcggagggc acgacctgag gctgccgagg aagaaggaga   600

tgtcgttcga gtggcacctg ttctcgtcca ccgtcgtccc cttgcagaag agtacgaggc   660

actacgtact ccgagacgtg ttggtgatgt gcgtcttctc ggagagggac agaggcccat   720

ttccacctcc accaccacgt gtctttcgcc ggcgtttttt tgagctcatt acctagg     777
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<210> 123

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker for peptide-FC linkage

<400> 123

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Gly Gly Gly Lys Gly Gly Gly Gly
1               5
```

<210> 124

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker for peptide-FC linkage

<400> 124

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Gly Gly Gly Asn Gly Ser Gly Gly
1               5
```

<210> 125

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker for peptide-FC linkage

<400> 125

Gly Gly Gly Cys Gly Gly Gly Gly
1   5

<210> 126

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker for peptide-FC linkage

<400> 126

Gly Pro Asn Gly Gly
1   5

<210> 127

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for PCR

<400> 127
cggcgcaact atcggtatca agctg   25

<210> 128

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for PCR

<400> 128
catgtaccgt aacactgagt ttcgtc   26

<210> 129

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 129
tatgattcat ggttgttggt ttacagaaga aggttgtgtt tggcaac   47

<210> 130

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 130
tcgagttgcc aaacacaacc ttcttctgta aaccaacaac catgaatca   49

<210> 131

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 131
tatgtatatg caatgtcaat ttgctcgtga tggttgtcca caatggc   47

<210> 132

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 132
tcgagccatt gtggacaacc atcacgagca aattgacatt gcatataca   49

<210> 133

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 133
tatgaaatta caatgtcaat attctgaatc tggttgtcca acaattc   47

<210> 134

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 134
tcgagaattg ttggacaacc agattcagaa tattgacatt gtaatttca   49

<210> 135

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 135
tatgtttta caatgtgaaa tttctggtgg tgcttgtcca gctccac   47

<210> 136

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 136
tcgagtggag ctggacaagc accaccagaa atttcacatt gtaaaaaca   49

<210> 137

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 137
tatgaaatta caatgtgaat tttctacttc tggttgtcca gatttac   47

<210> 138

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 138
tcgagtaaat ctggacaacc agaagtagaa aattcacatt gtaatttca   49

<210> 139

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 139
tatgaaatta caatgtgaat tttctactca aggttgtcca gatttac   47

<210> 140

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 140
tcgagtaaat ctggacaacc ttgagtagaa aattcacatt gtaatttca   49

<210> 141

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 141
tatgaaatta caatgtgaat tttctacttc tggttgtcct tggttac   47

<210> 142

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 142
tcgagtaacc aaggacaacc agaagtagaa aattcacatt gtaatttca   49

<210> 143

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 143
tatgattcaa ggttgttggt ttactgaaga aggttgtcct tggcaac   47

<210> 144

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 144
tcgagttgcc aaggacaacc ttcttcagta aaccaacaac cttgaatca   49

<210> 145

<211> 59

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 145
tatgtctttt gattgtgata atccttgggg tcatgtttta caatcttgtt ttggtttttc   59

<210> 146

<211> 61

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 146
tcgagaaaac caaacaaga ttgtaaaaca tgaccccaag gattatcaca atcaaaagac   60

a   61

<210> 147

<211> 59

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 147
tatgtctttt gattgtgata atccttgggg tcataaatta caatcttgtt ttggttttc   59

<210> 148

<211> 61

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 148
tcgagaaaac caaacaaga ttgtaattta tgaccccaag gattatcaca atcaaaagac   60

a   61

<210> 149

<211> 65

<212> DNA

<213>   Artificial Sequence

<220>

<223>   Oligonucleotide used in peptide construction

<400>   149
tatgacaggt tatacagaat atacagaaga atggccaatg ggttttggtt atcaatggtc   60

ctttc   65

<210>   150

<211>   67

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Oligonucleotide used in peptide construction

<400>   150
tcgagaaagg accattgata accaaaaccc attggccatt cttctgtata ttctgtataa   60

cctgtca   67

<210>   151

<211>   65

<212>   DNA

<213>   Artificial Sequence

<220>

<223>   Oligonucleotide used in peptide construction

<400>   151
tatgacagat tggttatctg attttccatt ctatgaacaa tactttggtt taatgccacc   60

tggtc   65

<210>   152

<211>   67

<212>   DNA

<213>   Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 152
tcgagaccag gtggcattaa accaaagtat tgttcataga atggaaaatc agataaccaa   60

tctgtca   67

<210> 153

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 153
tatgtttatg cgttttccta acccatggaa attagttgaa ccacctcaag gttggtacta   60

tggtc   65

<210> 154

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 154
tcgagaccat agtaccaacc ttgaggtggt tcaactaatt tccatgggtt aggaaaacgc   60

ataaaca   67

<210> 155

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 155
tatggttgtt aaagctccac attttgaatt cttagctcca cctcattttc atgaatttcc   60

atttc   65

<210> 156

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 156
tcgagaaatg gaaattcatg aaaatgaggt ggagctaaga attcaaaatg tggagcttta   60

acaacca   67

<210> 157

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 157
tatgttttct tatatttgga ttgatgaaac tccgtctaac attgatcgtt atatgctgtg   60

gctgc   65

<210> 158

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 158
tcgagcagcc acagcatata acgatcaatg ttagacggag tttcatcaat ccaaatataa   60

gaaaaca   67

<210> 159

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 159
tatggttaac tttccgaaag ttccggaaga tgttgaaccg tggccgtggt ctctgaaact   60

gtatc   65

<210> 160

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 160
tcgagataca gtttcagaga ccacggccac ggttcaacat cttccggaac tttcggaaag   60

ttaacca   67

<210> 161

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction .

<400> 161
tatgacttgg cacccgaaaa cttatgaaga atttgctctg ccgttttttg ttccggaagc   60

tccgc   65

<210> 162

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 162
tcgagcggag cttccggaac aaaaaacggc agagcaaatt cttcataagt tttcgggtgc   60

caagtca   67

<210> 163

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 163
tatgtggcat tttggtactc catatattca acaacaacca ggtgtttatt ggttacaagc   60

tccac   65

<210> 164

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 164
tcgagtggag cttgtaacca ataaacacct ggttgttgtt gaatatatgg agtaccaaaa   60

tgccaca   67.

<210> 165

<211> 65

<212> DNA

<210> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 165
tatggtttgg aattatggtc cattttttat gaattttcca gattctactt attttttaca   60

tgaac   65

<210> 166

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 166
tcgagttcat gtaaaaaata agtagaatct ggaaaattca taaaaaatgg accataattc   60

caaacca   67

<210> 167

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 167
tatgtggcgt attcattcta aaccattaga ttattctcat gtttggtttt ttccagctga   60

ttttc   65

<210> 168

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 168
tcgagaaaat cagctggaaa aaaccaaaca tgagaataat ctaatggttt agaatgaata   60

cgccaca   67

<210> 169

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 169
tatgttttgg gatggtaatc aaccaccaga tattttagtt gattggccat ggaatccacc   60

agttc   65

<210> 170

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 170
tcgagaactg gtggattcca tggccaatca actaaaatat ctggtggttg attaccatcc   60

caaaaca   67

<210> 171

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

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<400>  171
tatgttttat tctttagaat ggttaaaaga tcattctgaa ttttttcaaa ctgttactga        60

atggc                                                                    65


<210>  172

<211>  67

<212>  DNA

<213>  Artificial Sequence


<220>

<223>  Oligonucleotide used in peptide construction

<400>  172
tcgagccatt cagtaacagt ttgaaaaaat tcagaatgat cttttaacca ttctaaagaa        60

taaaaca                                                                  67


<210>  173

<211>  65

<212>  DNA

<213>  Artificial Sequence


<220>

<223>  Oligonucleotide used in peptide construction

<400>  173
tatgcaattt atggaattac tgaaattctt taattctcca ggtgattctt ctcatcactt        60

cttac                                                                    65


<210>  174

<211>  67

<212>  DNA

<213>  Artificial Sequence


<220>

<223>  Oligonucleotide used in peptide construction

<400>  174
tcgagtaaga agtgatgaga agaatcacct ggagaattaa agaatttcag taattccata        60

aattgca                                                                  67
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<210> 175

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 175
tatgactaat gttgattgga tttctaataa ttgggaacat atgaaatctt tttttactga   60

agatc   65

<210> 176

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 176
tcgagatctt cagtaaaaaa agatttcata tgttcccaat tattagaaat ccaatcaaca   60

ttagtca   67

<210> 177

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 177
tatgccaaat gaaaaaccat atcaaatgca atcttggttt ccaccagatt ggccagttcc   60

atatc   65

<210> 178

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<211>   67

<212>   DNA

<213>   Artificial Sequence


<220>

<223>   Oligonucleotide used in peptide construction
<400>   178
tcgagatatg gaactggcca atctggtgga aaccaagatt gcatttgata tggttttttca      60

tttggca                                                                  67


<210>   179

<211>   65

<212>   DNA

<213>   Artificial Sequence


<220>

<223>   Oligonucleotide used in peptide construction
<400>   179
tatgtggtct catactgaat gggttccaca agtttggtgg aaaccaccaa atcattttta      60

tgttc                                                                    65


<210>   180

<211>   67

<212>   DNA

<213>   Artificial Sequence


<220>

<223>   Oligonucleotide used in peptide construction
<400>   180
tcgagaacat aaaaatgatt tggtggtttc caccaaactt gtggaaccca ttcagtatga      60

gaccaca                                                                  67


<210>   181

<211>   65

<212>   DNA
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<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 181
tatggttcca tgggaacatg atcatgattt atgggaaatt atttctcaag attggcatat   60

tgctc   65

<210> 182

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 182
tcgagagcaa tatgccaatc ttgagaaata atttcccata aatcatgatc atgttcccat   60

ggaacca   67

<210> 183

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 183
tatggtttta catttacaag atccacgtgg ttggtctaat tttccaccag gtgttttaga   60

attac   65

<210> 184

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 184
tcgagtaatt ctaaaacacc tggtggaaaa ttagaccaac cacgtggatc ttgtaaatgt   60

aaaacca   67

<210> 185

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 185
tatgtggggt gaatggatta atgatgctca agttcacatg catgaaggtt ttatttctga   60

atctc   65

<210> 186

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used in peptide construction

<400> 186
tcgagagatt cagaaataaa accttcatgc atgtgaactt gagcatcatt aatccattca   60

ccccaca   67

<210> 187

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for PCR

<400> 187
acaaacaaac atatgggtgc acagaaagcg gccgcaaaaa aactcgaggg tggaggcggt   60

ggggaca   67

<210> 188

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for PCR

<400> 188
ggtcattact ggaccggatc   20

<210> 189

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used in PCR

<400> 189
cgtacaggtt tacgcaagaa aatgg   25

<210> 190

<211> 66

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used in PCR

<400> 190
tttgttggat ccattactcg agttttttttg cggccgcttt ctgtgcacca ccacctccac   60

ctttac   66

<210> 191

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used in PCR
<400> 191
caaacgaatg gatcctcatt aaagccaga   29

<210> 192

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used in PCR
<400> 192
ggtggtgcgg ccgcactcga gactgttgaa agttgtttag ca   42

<210> 193

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used in PCR
<400> 193
aacacaaaag tgcacagggt ggaggtggtg gtgcggccgc act   43

<210> 194

<211> 76

<212> DNA

<213> Artificial Sequence

<220>

<223>   Oligonucleotide for library preparation

<220>

<221>   misc_feature

<222>   (1)..(2)

<223>   N in positions 1-2 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (4)..(5)

<223>   N in positions 4-5 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (7)..(8)

<223>   N in positions 7-8 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (10)..(11)

<223>   N in positions 10-11 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (13)..(14)

<223>   N in positions 13-14 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (16)..(17)

<223>   N in positions 16-17 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (19)..(20)

<223> N in positions 19-20 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (28)..(29)

<223> N in positions 28-29 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (40)..(41)

<223> N in positions 40-41 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (43)..(44)

<223> N in positions 43-44 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (46)..(47)

<223> N in positions 46-47 can be any nucleotide A, G, C or T

<220>

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<222> (49)..(50)

<223> N in positions 49-50 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (52)..(53)

<223> N in positions 52-53 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (55)..(56)

<223> N in positions 55-56 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (58)..(59)

<223> N in positions 58-59 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<223> K in positions 3, 6, 9, 12, 15, 18, 21, 30, 42, 45, 48, 51, 54, 5
      7, and 60 represents an equal representation of nucleotides G and
       T

<400> 194
nnknnknnkn nknnknnknn kctgcagnnk sartwtagcn nknnknnknn knnknnknnk   60

cattctctcg agatca   76

<210> 195

<211> 91

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for library preparation

<220>

<221> misc_feature

<222> (16)..(17)

<223> N in positions 16-17 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (19)..(20)

<223> N in positions 19-20 can be any nucleotide A, G, C or T

<220>

<221> misc_feature ·

<222> (22)..(23)

<223> N in positions 22-23 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (34)..(35)

<223> N in positions 34-35 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (55)..(56)

<223> N in positions 55-56 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (67)..(68)

<223> N in positions 67-68 can be any nucleotide A, G, C or T

<220>

<221> misc_feature ·

<222> (70)..(71)

<223> N in positions 70-71 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (73)..(74)

<223> N in positions 73-74 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<223> K in positions 18, 21, 24, 36, 57 69, 72 and 75 represents an equ
       al representation of nucleotides G and T

<400> 195
cacagtgcac agggtnnknn knnkaaactg cagnnkgaat ttagcaccag cggcnnkccg   60

gatctgnnkn nknnkcattc tctcgagatc a   91

<210> 196

<211> 91

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for library preparation

<220>

<221> misc_feature

<222> (16)..(17)

<223> N in positions 16-17 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (19)..(20)

<223> N in positions 19-20 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (22)..(23)

<223> N in positions 22-23 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (25)..(26)

<223> N in positions 25-26 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (28)..(29)

<223> N in positions 28-29 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (31)..(32)

<223> N in positions 31-32 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (34)..(35)

<223> N in positions 34-35 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (55)..(56)

<223> N in positions 55-56 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (58)..(59)

<223> N in positions 58-59 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (61)..(62)

<223> N in positions 61-62 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (64)..(65)

<223> N in positions 64-65 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (67)..(68)

<223> N in positions 67-68 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (70)..(71)

<223> N in positions 70-71 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (73)..(74)

<223> N in positions 73-74 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<223> K in positions 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54
       , 57, 60, 63, 66, 69, 72 and 75 represents an equal representatio
       n of nucleotides G and T

<400> 196
cacagtgcac agggtnnknn knnknnknnk nnknnktgkt tkackgakga kggknnknnk   60

nnknnknnkn nknnkcattc tctcgagatc a   91

<210> 197

<211> 97

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for library preparation

<220>

<221> misc_feature

<222> (16)..(17)

<223> N in positions 16-17 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (79)..(80)

<223> N in positions 79-80 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<223> K in positions 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54
     , 57, 60, 63, 66, 69, 72, 75, 78 and 81 represents an equal repre
     sentation of nucleotides G and T

<400> 197
cacagtgcac agggtnnktt ktgkgakggk aakcakcckc ckgakatktt kgtkgaktgk   60

ccktgkaakc ckcckgtknn kcattctctc gagatca   97

<210> 198

<211> 97

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for library preparation

<220>

<221> misc_feature

<222> (16)..(17)

<223> N in positions 16-17 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222> (79)..(80)

<223> N in positions 79-80 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<223> K in positions 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54
      , 57, 60, 63, 66, 69, 72, 75, 78 and 81 represents an equal repre
      sentation of nucleotides G and T

<400> 198
cacagtgcac agggtnnkac kgaktgkctk agkgakttkc ckttktakga kcaktakttk   60

ggkctkatkc ckcckggknn kcattctctc gagatca   97

<210> 199

<211> 91

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for library preparation

<220>

<221> misc_feature

<222> (16)..(17)

<223> N in positions 16-17 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<222>   (19)..(20)

<223>   N in positions 19-20 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (22)..(23)

<223>   N in positions 22-23 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (34)..(35)

<223>   N in positions 34-35 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (55)..(56)

<223>   N in positions 55-56 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (67)..(68)

<223>   N in positions 67-68 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (70)..(71)

<223>   N in positions 70-71 can be any nucleotide A, G, C or T

<220>

<221>   misc_feature

<222>   (73)..(74)

<223>   N in positions 73-74 can be any nucleotide A, G, C or T

<220>

<221> misc_feature

<223> K in positions 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54
       , 57, 60, 63, 66, 69, 72 and 75 represents an equal representatio
       n of nucleotides G and T

<400> 199
cacagtgcac agggtnnknn knnkaakctk caknnkgakt tktckacktc kggknnkcck   60

gakctknnkn nknnkcattc tctcgagatc a   91

<210> 200

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for PCR

<400> 200
cacagtgcac agggt   15

<210> 201

<211> 16

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for PCR

<400> 201
tgatctcgag agaatg   16

<210> 202

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 202

Ala Gln Pro Thr Asp Gln Leu Gly Asp Trp Met Leu Asn Tyr Phe Arg
1   5   10   15

Leu Val Pro Pro Gly Thr
            20

<210> 203

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 203

Met Tyr Leu Asp Glu Trp Gln Trp Pro Pro Asp Val Phe Val Glu Trp
1   5   10   15

Pro Trp Lys Val Ser Val Asp
            20

<210> 204

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 204

Met Tyr Gln Lys Leu Gln Cys Glu Leu Ser Thr Ser Gly Cys Pro Asp
1   5   10   15

Leu Trp Arg Ala Leu Glu
            20

<210> 205

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 205

Ala Gln Leu Gln Ala Leu Leu Arg Glu Leu Pro Leu Tyr Glu Gln Phe
1   5   10   15

Phe Arg Leu Met Pro Pro Gly Tyr Leu Glu
            20   25

<210> 206

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 206

Ala Gln Val Thr Asn Ile Leu Ser Gln Leu Pro Leu Trp Gln Gln Trp
1   5   10   15

Leu Gly Leu Met Pro Pro Gly Val Leu Glu
            20   25

<210> 207

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura

lly occurring sequence

<400> 207

Met Ala Met Ala Gln Leu Gln Cys Glu Phe Ser Val Gln Gly Cys Pro
1   5   10   15

Ser Phe Val Leu Glu
          20

<210> 208

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 208

Met Leu His Asn Thr Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro
1   5   10   15

Asp Leu Pro Leu Gln Leu Glu
          20

<210> 209

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 209

Met Trp Gly Gln Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro
1   5   10   15

Asp Leu Pro Lys Ala Leu Glu
          20

<210> 210

<211>   21

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natura
        lly occurring sequence

<400>   210

Met Ile Asp Trp Leu Ser Gln Asn Arg Leu Phe Glu Gln Tyr Phe Glu
1   5   10   15

Leu Ile Pro Pro Gly
            20

<210>   211

<211>   23

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natura
        lly occurring sequence

<400>   211

Gln Pro Thr Asp Gln Leu Gly Asp Trp Met Leu Asn Tyr Phe Arg Leu
1   5   10   15

Val Pro Pro Gly Thr Leu Glu
            20

<210>   212

<211>   26

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natura
        lly occurring sequence

<400>   212

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Ala Gln Leu Ala Asp Leu Leu Ala Gln Leu Pro Met Trp Glu Gln Tyr
1               5                   10              15

Leu Gly Leu Thr Pro Pro Ser Ser Leu Glu
            20                  25
```

<210> 213

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 213

```
Ala Gln Leu Arg Glu Leu Leu Ser Asp Leu Pro Met Trp Glu Gln Tyr
1               5                   10              15

Phe Arg Leu Met Pro Pro Gly Tyr Leu Glu
            20                  25
```

<210> 214

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 214

```
Met Val Gln Arg Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro
1               5                   10              15

Asp Leu Thr Leu Leu Leu Glu
            20
```

<210> 215

<211> 21

```
<212>  PRT

<213>  Artificial Sequence


<220>

<223>  Therapeutically active peptide of randomly generated,  non-natura
       lly occurring sequence

<400>  215

Met Gly Pro Leu Val Leu Gln Cys Glu Phe Ser Gln Gly Gly Cys Pro
1               5                   10                  15

Thr Phe Leu Leu Glu
            20


<210>  216

<211>  24

<212>  PRT

<213>  Artificial Sequence


<220>

<223>  Therapeutically active peptide of randomly generated,  non-natura
       lly occurring sequence

<400>  216

Ala Glu Gln Ser Gln Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys
1               5                   10                  15

Pro Asp Leu Pro Gln Met Leu Glu
            20


<210>  217

<211>  25

<212>  PRT

<213>  Artificial Sequence


<220>

<223>  Therapeutically active peptide of randomly generated,  non-natura
       lly occurring sequence

<400>  217

Met His Met Ser Asp Val Tyr Trp Pro Pro Asp Val Phe Val Glu Trp
```

```
1                5                10                15

Pro Trp Val Pro Gln Val Pro Leu Glu
            20                25


<210>  218

<211>  25

<212>  PRT

<213>  Artificial Sequence


<220>

<223>  Therapeutically active peptide of randomly generated,  non-natura
       lly occurring sequence

<400>  218

Met Trp Val Gly Lys Gly Arg Leu Gln Cys Glu Ile Val Gly Gln Cys
1                5                10                15


Pro Gln Asn Pro Arg Trp Leu Leu Glu
            20                25


<210>  219

<211>  24

<212>  PRT

<213>  Artificial Sequence


<220>

<223>  Therapeutically active peptide of randomly generated,  non-natura
       lly occurring sequence

<400>  219

Ala Gln Pro Thr Asp Gln Leu Gly Asp Trp Met Leu Asn Tyr Phe Arg
1                5                10                15


Leu Val Pro Pro Gly Thr Leu Glu
            20


<210>  220

<211>  25

<212>  PRT
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<210> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 220

Met Pro Glu Trp Lys Gly Tyr Trp Pro Pro Glu Val Phe Ile Glu Trp
1   5   10   15

Pro Trp Ser Pro Pro Val Gln Leu Glu
            20   25

<210> 221

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 221

Pro Thr Asp Gln Leu Gly Asp Trp Met Leu Asn Tyr Phe Arg Leu Val
1   5   10   15

Pro Pro Gly Thr
            20

<210> 222

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 222

Met Ile Pro Gly Lys Leu Gln Cys Glu Leu Ser Ser Ser Gly Cys Pro
1   5   10   15

Asn Leu Gln Ala Met Leu Glu
          20

<210> 223

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
       lly occurring sequence

<400> 223

Met Asn Arg Met Gln Leu Gln Cys Glu Phe Ser Gln Ala Gly Cys Pro
1   5   10   15

Val Trp Ala Leu Glu
          20

<210> 224

<211> 24

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
       lly occurring sequence

<400> 224

Ala Gln Gln Ser Gln Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys
1   5   10   15

Pro Asp Leu Pro Leu Gln Leu Glu
          20

<210> 225

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 225

Ala Gln Gln Thr Glu Trp Leu Trp Ser Leu Pro Leu Val Glu Gln Tyr
1   5   10   15

Phe Ser Leu Val Pro Pro Gly Tyr Leu Glu
            20   25

<210> 226

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 226

Ala Gln Thr Gln Glu Trp Met Met Asn Leu Pro Leu Val Glu Gln Tyr
1   5   10   15

Phe Gly Leu Thr Pro Pro Gly Met Leu Glu
            20   25

<210> 227

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 227

Pro Thr Asp Gln Leu Gly Asp Trp Met Leu Asn Tyr Phe Arg Leu Val
1   5   10   15

Pro Pro Gly

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<210>  228

<211>  21

<212>  PRT

<213>  Artificial Sequence


<220>

<223>  Therapeutically active peptide of randomly generated,  non-natura
       lly occurring sequence

<400>  228

Met Asp Glu Trp Gln Trp Pro Pro Asp Val Phe Val Glu Trp Pro Trp
1               5                   10                  15


Lys Val Ser Val Asp
            20


<210>  229

<211>  25

<212>  PRT

<213>  Artificial Sequence


<220>

<223>  Therapeutically active peptide of randomly generated,  non-natura
       lly occurring sequence

<400>  229

Met Ser Trp Gln Glu Gly Met Trp Pro Pro Glu Val Phe Val Glu Trp
1               5                   10                  15


Pro Trp Thr Ala His Asp Trp Leu Glu
            20                  25


<210>  230

<211>  26

<212>  PRT

<213>  Artificial Sequence
```

<220>   .

<223>   Therapeutically active peptide of randomly generated,   non-natura
         lly occurring sequence

<400>   230

Ala Gln Gln Gly Met Trp Pro Gly Ala Met Ser Leu Leu Glu Gln Tyr
1   5   10   15

Phe Ala Leu Thr Pro Pro Gly Leu Leu Glu
            20   25

<210>   231

<211>.   18

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated,   non-natura
         lly occurring sequence

<400>   231

Asp Gln Leu Gly Asp Trp Met Leu Asn Tyr Phe Arg Leu Val Pro Pro
1   5   10   15

Gly Thr

<210>   232

<211>   22

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated,   non-natura
         lly occurring sequence

<400>   232

Met Ile Asp Trp Leu Ser Gln Asn Arg Leu Phe Glu Gln Tyr Phe Glu
1   5   10   15

Leu Ile Pro Pro Gly Val
            20

<210> 233

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 233

Met Ser Gly Asp Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro
1   5   10   15

Asp Leu Pro Ile Ser Leu Glu
            20

<210> 234

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 234

Met Gln Gln Gly Lys Leu Gln Cys Glu Leu Ser Thr Ala Gly Cys Pro
1   5   10   15

Glu Leu Leu Leu Pro Leu Glu
            20

<210> 235

<211> 24

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura

lly occurring sequence

<400> 235

Ala Gln Gln Ser Gln Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys
1   5   10   15

Pro Asp Leu Pro Leu Met Leu Glu
            20

<210> 236

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
       lly occurring sequence

<400> 236

Ala Gln Asn Pro Gly His Leu Leu Asp Leu Pro Leu Phe Tyr Gln Tyr
1   5   10   15

Phe Gln Leu Met Pro Pro Gly Ile Leu Glu
            20   25

<210> 237

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
       lly occurring sequence

<400> 237

Pro Thr Asp Gln Leu Gly Asp Trp Met Leu Asn Tyr Phe Arg Leu Val
1   5   10   15

Pro Pro Gly Thr Leu Glu
            20

<210> 238

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 238

Gln Thr Asp Trp Arg Trp Asp Leu Pro Phe Val Glu Asp Tyr Phe Arg
1   5   10   15

Leu Arg Pro Pro Gly Val
            20

<210> 239

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 239

Ala Gln Met Ile Asp Trp Leu Ser Gln Asn Arg Leu Phe Glu Gln Tyr
1   5   10   15

Phe Glu Leu Ile Pro Pro Gly Val Leu Glu
            20   25

<210> 240

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 240

```
Met Gln Leu Trp Asp Gly Lys Trp Pro Pro Glu Val Phe Val Glu Trp
1               5               10              15

Pro Trp Asn Pro Pro Val Gln
            20
```

<210> 241

<211> 24

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 241

```
Ala Gln Gln Ser Gln Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys
1               5               10              15

Pro Asp Leu Pro Gln Gln Leu Glu
            20
```

<210> 242

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 242

```
Met Val Glu Trp Gln Trp Cys Trp Phe Thr Glu Glu Gly Cys Pro Leu
1               5               10              15

Pro Leu Arg Leu Glu
            20
```

<210> 243

<211> 25

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated,   non-natura
        lly occurring sequence

<400>   243

Met Trp Leu Phe Glu Gly Gln His Pro Pro Glu Val Leu Val Glu Trp
1   5   10   15

Pro Trp Val Trp Pro Val Ala Leu Glu
          20   25

<210>   244

<211>   25

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated,   non-natura
        lly occurring sequence

<400>   244

Met Arg Tyr Phe Glu Gly Asn Trp Pro Leu Asp Val Phe Val Asp Trp
1   5   10   15

Pro Trp Asn Pro Thr Val Asp Leu Glu
          20   25

<210>   245

<211>   22

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated,   non-natura
        lly occurring sequence

<400>   245

Met Gln Val Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro Glu

1   5   10   15

Met His Arg Ile Leu Glu
            20

<210> 246

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 246

Met Gln Leu Gly Lys Leu Gln Cys Glu Leu Ser Thr Ala Gly Cys Pro
1   5   10   15

Asp Leu Pro Tyr Val Leu Glu
            20

<210> 247

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 247

Met Tyr Leu Asp Glu Trp Gln Trp Pro Pro Asp Val Phe Val Glu Trp
1   5   10   15

Pro Trp Lys Val Ser
            20

<210> 248

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 248

Met Thr Val Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro Asp
1   5   10   15

Leu Ala Trp Gln Leu Glu
            20

<210> 249

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 249

Met Phe Arg Tyr Gln Leu Gln Cys Glu Leu Ser Ser Ser Gly Cys Pro
1   5   10   15

Asp Leu Asn Asn Ile Leu Glu
            20

<210> 250

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 250

Ala Gln Ala Arg Glu Trp Gln Thr Glu Leu Pro Phe Phe Glu Gln Tyr
1   5   10   15

`

```
Phe Ala Leu Met Pro Pro Gly Val Leu Glu
            20                  25
```

```
<210>  251

<211>  22

<212>  PRT

<213>  Artificial Sequence


<220>

<223>  Therapeutically active peptide of randomly generated,  non-natura
       lly occurring sequence

<400>  251
```

```
Gln Thr Asp Trp Leu Ser Asp Leu Pro Leu Leu Glu Gln Tyr Phe Arg
1               5                   10                  15
```

```
Leu Met Pro Pro Gly Val
            20
```

```
<210>  252

<211>  21

<212>  PRT

<213>  Artificial Sequence


<220>

<223>  Therapeutically active peptide of randomly generated,  non-natura
       lly occurring sequence

<400>  252
```

```
Met Ser Gln Ala Pro Leu Gln Cys Glu Tyr Ser Ser Ser Gly Cys Pro
1               5                   10                  15
```

```
Leu Trp Gln Leu Glu
            20
```

```
.<210>  253

<211>  26

<212>  PRT

<213>  Artificial Sequence
```

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 253

Ala Gln Leu Thr Asp Gln Leu Arg Leu Leu Pro Leu Tyr Leu Gln Tyr
1   5   10   15

Phe Ser Leu Ile Pro Pro Val Thr Leu Glu
            20   25

<210> 254

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 254

Met Gln Ser Trp Asp Val Lys Trp Pro Pro Asp Val Phe Val Glu Trp
1   5   10   15

Pro Tyr Asn Pro Pro Ile Gln Leu Glu
            20   25

<210> 255

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 255

Met Ile Lys Gln Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro
1   5   10   15

Asp Leu Trp Met Ser Leu Glu

                20

<210>   256

<211>   23

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated,   non-natura
        lly occurring sequence

<400>   256

Met His Glu Gln Lys Leu Gln Cys Glu Leu Ser Thr Ser Gly Cys Pro
1   5   10   15

Asp Leu Val Gln Met Leu Glu
            20

<210>   257

<211>   22

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated,   non-natura
        lly occurring sequence

<400>   257

Met Gln Phe Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro Asp
1   5   10   15

Leu Arg His Pro Leu Glu
            20

<210>   258

<211>   26

<212>   PRT

<213>   Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 258

Ala Gln Met Gln Glu Leu Leu Arg Glu Leu Pro Leu Tyr Glu Gln Tyr
1   5   10   15

Met Ala Leu Met Pro Pro Gly Met Leu Glu
            20   25

<210> 259

<211> 24

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 259

Ala Gln Gln Gln Gln Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys
1   5   10   15

Pro Asp Leu Pro Leu Met Leu Glu
            20

<210> 260

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 260

Ala Gln Gln Thr Asn Trp Cys Met Gly Ile Pro Tyr Cys Glu Gln Tyr
1   5   10   15

Phe Gly Leu Ser Pro His Gly Ile Leu Glu
            20   25

<210> 261

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 261

Met Ala Ser Leu Thr Leu Gln Cys Glu Tyr Ser Gly Gln Gly Cys Pro
1   5   10   15

Lys Trp Pro Leu Glu
            20

<210> 262

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 262

Ala Gln Leu Ala Glu Trp Leu Gln Gln Ile Pro Leu Tyr Glu Gln Tyr
1   5   10   15

Phe Gly Leu Met Pro Pro Asp Leu Leu Glu
            20   25

<210> 263

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura

lly occurring sequence

<400> 263

Met Glu Leu Ser Ala Arg Asn Trp Pro Pro Glu Ile Phe Glu Asp Trp
1   5   10   15

Pro Trp Gln Leu Pro Val Asp Leu Glu
            20   25

<210> 264

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated,   non-natura
      lly occurring sequence

<400> 264

Met Trp Met Thr Lys Leu Gln Cys Glu Phe Ser Ser His Gly Cys Pro
1   5   10   15

Gln Leu Thr Ser Met Leu Glu
            20

<210> 265

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated,   non-natura
      lly occurring sequence

<400> 265

Ala Glu Val Glu Trp Gln Trp Cys Trp Phe Thr Glu Glu Gly Cys Pro
1   5   10   15

Leu Pro Leu Arg Leu Glu
            20

<210> 266

<211>   25

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natura
         lly occurring sequence

<400>   266

Met Tyr Leu Asp Glu Trp Gln Trp Pro Pro Asp Val Phe Val Glu Trp
1   5   10   15

Pro Trp Lys Val Ser Val Asp Leu Glu
            20   25

<210>   267

<211>   23

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natura
         lly occurring sequence

<400>   267

Met Gln Ser Asn Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys Pro
1   5   10   15

Glu Leu Leu Asp Leu Leu Glu
            20

<210>   268

<211>   23

<212>   PRT

<213>   Artificial Sequence

<220>

<223>   Therapeutically active peptide of randomly generated, non-natura
         lly occurring sequence

<400>   268

```
Met Asn Val Gly Lys Leu Gln Cys Glu Leu Ser Thr Trp Gly Cys Pro
1               5               10              15

Val Pro Val Gln Gly Leu Glu
            20
```

<210> 269

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 269

```
Met Tyr Leu Trp Glu Gly Ile Trp Pro Ala Glu Val Phe Arg Glu Trp
1               5               10              15

Pro Trp Lys Pro Pro Asn Arg Leu Glu
        20              25
```

<210> 270

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
      lly occurring sequence

<400> 270

```
Met Leu Phe Trp Gln Gly Asn Pro Pro Pro Asp Val Phe Val Glu Trp
1               5               10              15

Pro Trp Gln Leu Pro Ala Ser Leu Glu
        20              25
```

<210> 271

<211> 26

<210> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 271

Ala Gln Asn Gly Asp Trp Met Arg Gly Leu Pro Phe Leu Glu Gln Tyr
1   5   10   15

Phe Gln Leu Leu Pro Pro Gly Val Leu Glu
            20   25

<210> 272

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natura
lly occurring sequence

<400> 272

Ala Gln Pro Thr Asp Gln Leu Gly Asp Trp Met Leu Asn Tyr Phe Arg
1   5   10   15

Leu Val Pro Pro Gly Thr Leu
            20

<210> 273

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Therapeutically active peptide of randomly generated, non-natural
ly occurring sequence

<400> 273

Trp Asp Met Cys His Phe Ser His Ala Ala Lys Leu Gln Ser Cys Phe

```
1               5                    10                      15
```

Pro His

```
.
```

<210> 274

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Consensus sequences from therapeutically active peptide of random
       ly generated, non-naturally occurring sequence

<220>

<221> misc_feature

<222> (1)..(1)

<223> X in position 1 is equal to any amino acid.

<220>

<221> misc_feature

<222> (10)..(12)

<223> X in positions 10, 11 and 12 is equal to any amino acid.

<220>

<221> misc_feature

<222> (4)..(4)

<223> X at position 4 is equal to F or W.

<220>

<221> misc_feature

<222> (5)..(5)

<223> X at position 5 is equal to S or T.

<400> 274

```
Xaa Cys Trp Xaa Xaa Glu Glu Gly Cys Xaa Xaa Xaa
1               5                   10
```

<210> 275

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Consensus sequences from therapeutically active peptide of random
      ly generated, non-naturally occurring sequence

<220>

<221> MISC_FEATURE

<222> (4)..(4)

<223> X in position 4 is equal to any amino acids.

<220>

<221> MISC_FEATURE

<222> (5)..(5)

<223> X at position 5 is equal to F or Y.

<220>

<221> MISC_FEATURE

<222> (7)..(8)

<223> X in positions 7 and 8 is equal to any amino acid.

<220>

<221> MISC_FEATURE

<222> (12)..(13)

<223> X in positions 12 and 13 is equal to any amino acid.

<400> 275

```
Leu Gln Cys Xaa Xaa Ser Xaa Xaa Gly Cys Pro Xaa Xaa
1               5                   10
```

```
<210>   276

<211>   18

<212>   PRT

<213>   Artificial Sequence


<220>

<223>   Consensus sequences from therapeutically active peptide of random
        ly generated, non-naturally occurring sequence

<220>

<221>   MISC_FEATURE

<222>   (1)..(3)

<223>   X in positions 1 through 3 is equal to any amino acid.


<220>

<221>   MISC_FEATURE

<222>   (5)..(11)

<223>   X in positions 5 through 11 is equal to any amino acid.


<220>

<221>   MISC_FEATURE

<222>   (16)..(18)

<223>   X in positions 16 through 18 is equal to any amino acid.


<400>   276

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Gln Ser Cys Xaa
1                5                   10              15

Xaa Xaa


<210>   277

<211>   14

<212>   PRT
```

<213> Artificial Sequence

<220>

<223> Peptide of randomly generated, non-naturally occurring sequence

<400> 277

Ile His Gly Cys Trp Phe Thr Glu Glu Gly Cys Val Trp Gln
1   5   10

<210> 278

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide of randomly generated, non-naturally occurring sequence

<400> 278

Leu Gln Met Cys Trp Phe Thr Glu Lys Gly Cys Glu Val Pro
1   5   10

<210> 279

<211> 24

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide of randomly generated, non-naturally occurring sequence

<400> 279

Ala Gln Gln Gln Gln Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys
1   5   10   15

Pro Asp Leu Pro Leu Met Leu Glu
            20

<210> 280

<211> 24

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide of randomly generated, non-naturally occurring sequence

<400> 280

Ala Gln Gln Ser Gln Lys Leu Gln Cys Glu Phe Ser Thr Ser Gly Cys
1   5   10   15

Pro Asp Leu Pro Gln Met Leu Glu
            20

<210> 281

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR forward primer to amplify phage clones

<400> 281
gttagctcac tcattaggca c   21

<210> 282

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR reverse primer to amplify phage clones

<400> 282
gtaccgtaac actgagtttc g   21

<210> 283

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer used to sequence amplified inserts of phage clones

<400> 283
gtaccgtaac actgagtttc g   21

<210> 284

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker for peptide-FC linkage

<400> 284

Gly Gly Gly Gly
1

<210> 285

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker for peptide-FC linkage

<400> 285

Gly Gly Gly Gly Gly
1   5

<210> 286

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker for peptide-FC linkage

<400> 286

Gly Gly Gly Gly Gly Gly Gly
1   5

A.	CLASSIFICATION OF SUBJECT MATTER
IPC(7)	: A61K 38/10, 38/16; C07H 21/04; C07K 7/08, 14/00
US CL	: 514/12, 13, 14; 530/324, 325, 326, 327; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/12, 13, 14; 530/324, 325, 326, 327; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99/53055 A2 (UNIVERSITY OF BRISTOL) 21 October 1999 (21/10/99), see entire document, especially claims 1 and 10.	1-16, 23-38, 45-60, 67-133, 141-151
A	WO 01/78698 A2 (WARNER-LAMBERT COMPANY) 25 October 2001 (25/10/01), see entire document, especially page 5, lines 18-26, and page 8, line 14 - page 9, line 3.	1-16, 23-38, 45-60, 67-133, 141-151

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier application or patent published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 2004 (13.01.2004)	09 FEB 2004
Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450	Jeffrey E. Russel
Facsimile No. (703)305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

Continuation of B. FIELDS SEARCHED Item 3:
EAST, DERWENT, DIALOG, STN, GENESEQ, SWISSPROT, PIR
search terms: nerve growth factor, bind, block, agonist, antagonist, inhibit, pain, amino acid sequences

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/29866

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claim Nos.: 17-22, 39-44, 61-66, 134-140, and 152-157
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐   The additional search fees were accompanied by the applicant's protest.

☐   No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)